Speciation and biogeography of heliconiine butterflies

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A thesis submitted for the degree of Doctor of Philosophy

of University College London

Genetics, Evolution and Environment

University College London

September 2012

Abstract

In this thesis I investigate the speciation and biogeography of neotropical heliconiine butterflies (Lepidoptera: Nymphalidae: Heliconiina). In Chapter 2, I present a large database of locality records for heliconiine species and subspecies, and use these data to test evolutionary and biogeographic hypotheses for their diversification. I find evidence that geographical gradients in species richness are driven at least in part by variation in speciation and/or extinction rates, rather than via evolutionary age or niche conservatism alone. The eastern Andes are characterised by high species richness and short phylogenetic branch lengths, suggesting that new species frequently arise there. Conversely, the Amazon basin is notable for high intra-specific phenotypic diversity. In Chapter 3, I use the geographic data to estimate the frequency of sympatric speciation in heliconiines. I find that the patterns of range overlap observed in heliconiines are consistent with sympatric speciation. However, parapatric speciation followed by a tendency for daughter species to expand rapidly into one another's ranges presents a plausible alternative explanation. I also present evidence that shifts in mimetic wing colour patterns and host plants are associated with speciation in heliconiines, suggesting that ecological adaptation may be important in triggering speciation events. In Chapter 4, I test the prediction that hybrid zones between Andean and Amazonian races of *Heliconius* should be moving towards the Andes. I find the position of the hybrid zones to be unchanged from 1986 – 2011, and located on a band of peak rainfall at the edge of the Andes. This suggests that rainfall peaks act as "sinks" for dispersal in butterflies and stabilise the hybrid zones on this low fitness region. The results oppose the Pleistocene Refugium theory, which

predicts that centres of ranges, rather than contact zones at the edges, should be centred on current rainfall peaks.

Acknowledgements

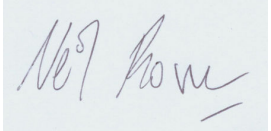
Three people have been critical to the completion of this thesis. First and foremost, I would like to thank Jim Mallet, both for his excellent supervision throughout my PhD, and for giving me this wonderful opportunity in the first place. Secondly, I thank Ally Phillimore for providing the perfect second supervisor; without his insight, methods and knowledge I would have been lost on many occasions. Finally, Kanchon Dasmahapatra has essentially acted as my unofficial third supervisor; I am extremely grateful to him for frequently discussing my work with me and for providing numerous lucid explanations. In addition, I am indebted to a number of other people that have provided me with assistance during the course of the PhD. I thank Keith Willmott for hosting me in Florida, for providing me with geographic data, for responding to my many queries on heliconiines and biogeography, and for his input into Chapter 2. Blanca Huertas greatly facilitated my visits to the Natural History Museum in London, provided me with an essential "toolbox" of taxonomic resources for heliconiines and also provided valuable input into Chapter 2. The following people contributed geographic data for mapping heliconiine distributions for which I am very grateful: Andy Brower, Dan Janzen, Chris Jiggins, José Luis Salinas-Gutiérrez and Jason Hall. I also thank the numerous individuals who helped in databasing heliconiine specimens at multiple institutions as part of the Tropical Andean Butterfly Diversity Project. I thank Rodolphe Bernard and Julian Rosser for their teaching and assistance with GIS, programming and computing. Marianne Elias provided helpful discussion with respect to testing the association between shifts in ecology and speciation. I thank Andrei Sourakov for making me so welcome in the McGuire Centre in Florida, and I thank Evren Akkaya, César López, Claire Mérot and Ronald

Mori Pezo for their assistance and company in the field in Peru. Finally, I thank Kanchon and Fraser Simpson for their good company throughout my PhD.

Declaration

I, Neil Rosser, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis. Chapter 2 has already been published in a slightly modified form in the Biological Journal of the Linnean Society. The co-authors include my primary and secondary supervisors (Prof. James Mallet, UCL and Dr. Ally Phillimore, Imperial College London) and colleagues who provided access to museum collections (Blanca Huertas, The Natural History Museum London and Keith Willmott, University of Florida).

Neil Rosser



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Chapter 1. Introduction

Biodiversity is unevenly distributed across the earth: for the majority of groups the tropics hold the majority of species, with the neotropics the most diverse region of all (Gaston and Hudson 1994, Thomas 1999, Orme et al. 2005). In this thesis I investigate the speciation and biogeography of neotropical heliconiine butterflies, with the aim of characterising the geographic and ecological contexts surrounding the evolution of races and species. Here, I briefly introduce the topic of the geography of speciation, and then, in light of this, review the biogeography of tropical America, focusing in particular on heliconiines.

The geography of speciation

Historically, three broad geographic modes of speciation have been recognised. Allopatric speciation occurs when diverging populations are geographically disjunct, parapatric speciation comprises a situation where geographically contiguous populations diverge and speciate, and sympatric speciation occurs when the diverging populations overlap geographically (Poulton 1904, Mayr 1942, Smith 1955). Recently, models of speciation based on population genetics have led to a growing emphasis on "potential for gene flow" or degree of gene flow rather than biogeographic context (Coyne and Orr 2004). This focus on gene flow highlights the somewhat arbitrary delineation of the biogeographic categories, and has led to debate concerning their merit (Butlin et al. 2008, Fitzpatrick et al. 2008, Mallet et al. 2009). In this thesis, I have adopted the more traditional, biogeographic view of speciation, as the newer, population genetic bases definitions do not address the classical debate as to whether geographic isolation is required for speciation (Mallet et al. 2009). Although I do not explicitly estimate gene flow, sympatric heliconiine species

certainly do encounter one another at appreciable frequencies, even when there is some spatial structure generated by habitat choice (Estrada and Jiggins 2002).

The geography of speciation has a long and controversial history stretching back to Darwin, who apparently pondered scenarios similar to the allopatric, parapatric and sympatric models discussed today (Stauffer 1975). A central problem concerns whether speciation usually requires diverging populations to be geographically isolated (Mayr 1963); while allopatric and parapatric speciation are generally accepted, sympatric speciation has been the subject of intense debate (Coyne and Orr 2004, Bolnick and Fitzpatrick 2007). Arguments against the existence and prevalence of sympatric speciation are founded on two lines of reasoning: one theoretical and the other empirical. Theoretical considerations of the genetic basis of sympatric speciation have shown that the conditions under which it can occur are much less permissive than allopatric speciation (Mayr 1963, Felsenstein 1981, Dieckmann and Doebeli 1999, Gavrilets and Waxman 2002, Gavrilets 2004). In particular, models of sympatric speciation have to deal with the problem of interbreeding between populations under divergent selection, which breaks up the evolving gene complexes that produce reproductive isolation (Felsenstein 1981). Biogeographical evidence that sympatric speciation is uncommon dates back to the observations of early naturalists that closely related pairs of animal species are usually allopatric (Jordan 1905, Jordan and Kellogg 1907, Mayr 1963, Coyne and Price 2000, Kisel and Barraclough 2010). Nonetheless, a number of case studies have been documented where sympatric speciation appears the most plausible explanation (Schliewen et al. 1994, Sorenson et al. 2003, Barluenga et al. 2006, Savolainen et al. 2006). In light of this, biologists now tend to debate how common the process actually is, rather than about its existence (Jiggins 2006).

Biogeography in the neotropics

The tendency for species richness to increase towards the equator is one of the most striking and consistent patterns in the natural world, but a widely accepted and general explanation remains elusive (Willig et al. 2003, Hillebrand 2004, Mittelbach et al. 2007). There is also substantial variation in species richness within the tropics, with the neotropics comprising the most biologically diverse region for most taxa (Gaston and Hudson 1994, Thomas 1999, Orme et al. 2005). A variety of hypotheses have been proposed as explanations for neotropical diversity (Haffer 2008). Usually these hypotheses have tended to focus on paleogeography or climate, but environmental and ecological conditions have also been invoked as important. The hypotheses are not mutually exclusive, and many are tightly interwoven. Many are discussed in terms of allopatric speciation, but parapatric speciation is also considered.

Paleogeographic hypotheses focus on the idea that geologic changes during the earth's history have resulted in the disjunction of populations and their subsequent speciation. Incursions by the sea into low lying areas of the South American continent during the Paleogene and Neogene due to tectonic and/or climatic changes have been hypothesised to fragment species distributions and cause speciation (Emsley 1963, Webb 1995). Similarly, the "lake model" proposes that the huge, freshwater lakes that formed in the Amazon basin from 23-7ma promoted allopatric speciation (Marroig and Cerqueira 1977, Hoorn et al. 2010). The geological arches (macro-scale geomorphic features) dividing Amazonian into sub-basins are also proposed to have isolated species geographically and added to environmental heterogeneity across geologic time (Patton and da Silva 1998, Hoorn and Wesselingh 2011). Recently, much attention has focused on Andean orogeny as the ultimate factor shaping South American biodiversity. Andean uplift seems likely to have had a range of

consequences for biota, including driving the lake model, splitting populations allopatrically and providing diverse environmental conditions that will promote speciation (Elias et al. 2009, Hoorn et al. 2010).

However, the hypothesis for Amazonian diversification to receive most attention during the past half century has been the Pleistocene Refugium theory, which posits that dry periods during the Pleistocene resulted in expansion of savannah ecosystems and the fragmentation of forests in Amazonia into "refugia" (areas where rainfall remained sufficiently high to maintain forests) (Haffer 1969; Haffer 1982; Brown et al. 1974; Brown 1979; Fox 1949; Turner 1965). A major problem for the refugium theory is that evidence from pollen sediments is equivocal as to whether the forests did in fact fragment during the most recent interglacials, although species composition does appear to have been altered and the climate considerably drier (Colinvaux et al. 1996, Hooghiemstra and van der Hammen 1998, Haberle and Maslin 1999, van der Hammen and Hooghiemstra 2000, Maslin and Burns 2000). In response to this, Colinvaux (1998) focused on the importance of changes in temperature and carbon dioxide levels rather than rainfall. He hypothesised that changes in these variables would be most pronounced in elevated areas, leading to geographically isolated "islands" of different climatic conditions and promoting allopatric speciation. More recently, the canopy-density hypothesis proposed that climatic variation did not result in the loss of forest cover, but did alter canopy structure sufficiently to fragment biotas and allow allopatric speciation (Cowling et al. 2001).

A further problem for the refugium theory is that a number of studies have shown existing South American biodiversity to predate the Pleistocene period for which it was originally proposed (Moritz et al. 2000, Wilf et al. 2003, Jaramillo et al. 2006,

Rull 2008), resulting in its modification to refer to the Paleogene, Neogene and Quaternary, and with an emphasis on Milankovitch cycles (Haffer 2008). However, a number of other lines of evidence also contradict the refugium theory. Nelson et al. (1990) showed that areas of endemism and the refugia they supposedly correspond to are correlated with sampling intensity and therefore may represent artefacts. Recently, species pairs across a suture zone between proposed refugia in northern Peru were shown to exhibit highly variable divergence times, contrary to the expectations if the suture zone was the product of secondary contact following expansion from refugia (Whinnett et al. 2005, Dasmahapatra et al. 2010).

A further class of climate-based hypotheses proposes that climatic changes result in areas of environmental stability and instability, and that such regions lead to speciation (Bush 1994, Fjeldså 1994, Fjeldså et al. 1999, Carnaval et al. 2009). Fjelda's "species pump / museum" hypothesis (Fjeldså 1994, Fjeldså et al. 1999) suggested that new bird lineages arise in Andean valleys and plateaus that are buffered from climatic fluctuations sufficiently that new lineages can arise, whereas the Amazonian lowlands were suggested to be unstable and characterised by frequent extinctions. In contrast, Bush (1994) proposed that speciation occurred in climatically unstable areas on the flanks of uplands. The instability was supposed to lead to range fragmentation via local extinctions, and ultimately speciation. The hypothesis is therefore related to the intermediate disturbance hypothesis (Connell 1978, Hubbell 1979) and environmental heterogeneity model (Gentry 1989).

Other explanations for speciation in Amazonia have focused on the importance of its enormous river system. The river barrier hypothesis posits that the Amazon and its tributaries act as barriers to migration, isolating populations (Wallace 1852, Ayres and

Clutton-Brock 1992, Colwell 2000, Gascon et al. 2000, Ribas et al. 2011). The river barrier hypothesis and the refugium hypothesis have also been combined to form the "river-refuge" hypothesis. This hypothesis supposes that arid periods in the earth's history would have resulted in the loss of forest in river headwaters, causing species ranges to retract to the lower reaches where they became isolated by the wide rivers (Haffer 1992, 1993).

The above hypotheses have almost invariably been discussed in the context of allopatric speciation, presumably because has been the most widely accepted mode of speciation during their development (Mayr 1963). However, a more recent model for explaining the biodiversity of tropical America is the environmental gradient hypothesis, which focuses on the importance of parapatric speciation (Endler 1977). Here, divergent selection to different environmental conditions across tropical America is proposed to drive speciation, which is uninhibited by gene flow due to the vast area of Amazonia (Endler 1977; Endler 1982; see also Smith et al. 1997).

The biogeography of heliconiine butterflies

Heliconius butterflies and their allies form a subtribe of neotropical butterflies (Nymphalidae: Heliconiina) comprising some 80 species and 452 subspecies (Lamas 2004). The *Heliconius* genus in particular are frequently used in evolutionary and ecological studies (Brown, 1981; Turner, 1981; Mallet & Joron, 1999; Dasmahapatra *et al.*, 2012). *Heliconius* are well known among natural historians for their aposematic wing coloration and participation in Müllerian mimicry rings, where species exhibit similar colour patterns thereby sharing the cost of educating predators as to their bad taste (Joron and Mallet 1998). Shifts in mimetic colour pattern have been implicated in triggering speciation, because *Heliconius* mate assortatively using colour pattern,

yielding "premating isolation", and because hybrids between colour patterns are usually poor mimics, giving "postmating isolation" (Jiggins et al. 2001, Mavárez et al. 2006, Chamberlain et al. 2009, Merrill et al. 2011a, 2011b). In some species mate preference is genetically correlated with the locus determining colour pattern (Kronforst et al. 2006, Chamberlain et al. 2009, Merrill et al. 2011b). *Heliconius* are therefore good candidates for sympatric speciation, as colour pattern shifts confer reproductive isolation and the link between the two is not disrupted by recombination (Felsenstein 1981). In addition, heliconiines are phytophagous insects, which have long been thought of as prone to sympatric speciation. This is because phytophagous insects frequently exhibit host plant fidelity and mate on their hosts, therefore reproductive isolation may arise after a host shift (Bush 1969).

There is considerable geographic variation in species richness of heliconiines, with diversity peaking in the eastern Andes close to the Equator (Chapter 2). Many heliconiines also exhibit marked geographic variation in colour pattern with some species comprising as many as 30 subspecies (Lamas 2004). These attributes make *Heliconius* ideal subjects for investigating geographic patterns of evolution in the neotropics. Bates (1863) provided the first biogeographical study of *Heliconius*, and linked variation in colour pattern along the Amazon river with speciation. Since then, multiple attempts to explain the geographic patterns of diversity have been made. Emsley (1963) proposed that speciation in heliconiines was a consequence of tectonic activity, with events such as emergence of the Andes and marine incursions dividing heliconiine populations and allowing allopatric speciation. Turner (1965) then proposed the refugium theory for heliconiines, generating a large body of work linking heliconiine races and areas of endemism to proposed refugia (Turner 1971; Turner 1976; Brown 1976; Brown 1979; Brown 1981; Brown 1982; Brown 1987a;

Brown 1987b; Brown 1987c; Brown et al. 1974; Lamas 1982). More recently, the environmental gradient hypothesis has been applied to *Heliconius* (Endler 1977, 1982, Benson 1982). Unfortunately, both the refugia and the environmental gradient hypotheses are predicted to produce very similar geographical patterns and distinguishing between the two is difficult (Endler 1977). However, some insight may be gained from the estimated divergence times of *Heliconius* races across a suture zone in Peru, which are scattered rather than clumped as expected if races were the product of isolation in Pleistocene refugia (Dasmahapatra et al. 2010).

A particular problem for *Heliconius* biologists is explaining the rampant geographic variation in colour patterns that characterises many of the species. Warning colour patterns are subject to strong frequency dependent, stabilising selection; colour patterns are favoured by selection when common enough to be recognized by predators (Mallet and Singer 1987). Therefore, the evolution of new colour patterns presents a paradox. Brown, Sheppard and Turner suggested that species gain new colour patterns via "biotic drift"(Brown et al. 1974, Turner 1983, 1984, Sheppard et al. 1985, Turner and Mallet 1996). In this model, stochastic variation in the species composition of local communities within a metapopulation leads to different species (and thus different colour patterns), being locally common in different areas. Rare species in the community will therefore be selected to mimic the locally predominant colour pattern. Although the biotic drift model is not tied to allopatry, it has been argued that its effects would be most profound within the refugium theory framework, with novel species assemblages likely to arise in small forest refugia (Turner 1982). The biotic drift model comprises a plausible hypothesis as to how selection could maintain the infra- and intra specific diversity of colour patterns in *Heliconius*. However, it cannot explain the evolution of entirely new patterns.

Mallet proposed that novel colour patterns in *Heliconius* could be explained by a version of Sewall Wright's shifting balance theory (Wright 1932, 1977, Mallet 1986a, 1986b, 1993, Mallet and Singer 1987, Mallet et al. 1990). Here, new colour patterns become established in local populations following stochastic events (such as drift following a reduction in selection pressure) which occasionally allow the new forms to surpass the critical frequency imposed by frequency-dependent selection and then be driven to fixation. The colour pattern may then spread out to other populations if there is any asymmetry in selection or migration between the two patterns (Barton 1979, Mallet and Barton 1989), or even in the absence of any selective imbalance if one of the new colour pattern is completely dominant (Mallet 1986a). Recently, molecular evidence and moving colour pattern clines have supported components of this shifting-balance type process hypothesis (Blum 2002, Hines et al. 2011). What selective benefits could new colour patterns confer? One possibility is that new colour patterns are in some way more effective at warning predators, and it has been suggested that different colour patterns may be more easily recognised by predators in different habitats (Benson 1982). For example, in the savannahs of northern South America the local race of *Heliconius erato* is black with a red forewing band, but in forested areas is black with yellow forewing bands, red patches on the bases on the forewings and red rays on the hindwings (Benson 1982, Blum 2008). In central America, the sister species *Heliconius cydno* (black with white forewing bands and white hindwing margin) and *Heliconius melpomene* (black with red forewing bands and a yellow hindwing bar) are associated with different forest types; *H. cydno* is typical of closed-canopy forest and whereas *H. melpomene* is common in more open, secondary forest (Estrada and Jiggins 2002).

Another notable biogeographic feature of *Heliconius* is that species hybrid zones and range boundaries for races are often associated with rivers, in particular the Amazon itself (Brown 1979, Lamas 1982). Two interpretations for such a pattern are possible. Firstly, large rivers may act as a partial or complete barrier to dispersal and allow allopatric or parapatric differentiation as in the river barrier hypothesis. Secondly, if the rivers represent partial barriers to dispersal they may trap moving colour pattern clines (Barton 1979). Either way, the presence of hybrid zones along the rivers has been argued to be evidence against the refugium hypothesis having operated in *Heliconius*, because gallery forest is likely to have persisted along rivers during dry periods and so these areas should form centres of endemism rather than hybrid zones (Mallet 1993). The Andes may also play a role in the diversification of *Heliconius*; hybrid zones are often found near mountains (Mallet 1993), and recently a number of recently diverged species closely allied to *Heliconius cydno* have been documented throughout the northern Andes (Mallet 2009).

Range boundaries between subspecies and species frequently occur in the isthmus of Panama, though the reason for this is not clear. One explanation could be area forms the point of contact between Central American and South American faunas following the closing of the Panama isthmus during the Pliocene at ~3.5Ma (Coates et al. 1992). However, many of the forms that meet there are probably younger than this (e.g. races of *Heliconius* (Dasmahapatra et al. 2010)). Alternatively, the isthmus may act as a partial barrier to dispersal, trapping mobile clines (Mallet 1993). This latter hypothesis seems unlikely, however, given the existence of mobile clines and hybrid zones travelling across it at present (Mallet 1993, Turner and Mallet 1996, Blum 2002, Dasmahapatra et al. 2002). Curiously, few heliconiines have colonised the Caribbean (Chapter 2). While those that have show some significant genetic divergence on

certain islands, none appear to have speciated and no new colour patterns have arisen (Holzinger & Holzinger 1994; Davies & Bermingham 2002). The lack of diversification may simply reflect recent colonisation of the islands, although it appears that some forms have been isolated for considerable amount of time. For example, *Heliconius charithonia simulator* on Jamaica is thought to have been isolated for approximately one million years (Davies and Bermingham 2002). The lack of new colour patterns on the islands may reflect low species richness of heliconiines, with few pre-existing mimicry rings available to radiate on to, and processes such as biotic drift unable to operate. However, that the Caribbean species are more or less monotypic could also be seen as evidence against phase 1 of the shifting balance, because random factors might well be expected to operate on small islands. For instance, islands typically have a lower diversity of predators and smaller predator population sizes that might be more susceptible to local extinctions, thus reducing selection and allowing the establishment of novel mutations. On the other hand, ecological release of the species that have colonised the islands means that effective population densities may be higher than on the mainland. Thus predators may select more strongly against variants, preventing drift from generating new colour patterns.

Aims

In Chapter 2, I present a large database of 58,059 point locality records for heliconiine species and subspecies compiled from museum specimens. I use these data to test evolutionary and biogeographic hypotheses for the diversification of the heliconiines. I also present range maps for 70 species and 434 subspecies of heliconiines. In Chapter 3, I use the geographic data to estimate the frequency of sympatric speciation in heliconiines using a recently developed comparative method (Phillimore et al.

2008), and I test whether mimicry shifts and host plant shifts are associated with speciation events and range overlap. In Chapter 4, I present a fine-scale case study of hybrid zones between co-mimetic races of *Heliconius erato* and *Heliconius melpomene* in northern Peru. I compare the position and shape of the hybrid zones from 1986 – 2011, and I characterise the environmental conditions associated with the hybrid zones. In Chapter 5, I summarise my principal findings and where possible draw conclusions.

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Chapter 2. Testing historical explanations for gradients in species richness in heliconiine butterflies of tropical America.

Abstract

I compiled a database of 58,059 point locality records for 70 species and 434 subspecies of heliconiine butterflies and used these data to test evolutionary hypotheses for their diversification. In order to study geographic patterns of diversity and contact zones, I mapped i) species richness, ii) mean molecular phylogenetic terminal branch length, iii) subspecies richness and the proportion of specimens that were subspecific hybrids, and iv) museum sampling effort. Heliconiine species richness is high throughout the Amazon region and peaks near the equator in the foothills and middle elevations of the eastern Andes. Mean phylogenetic terminal branch length is lowest in the eastern Andes and tends to be low in species rich areas. In contrast, areas of high subspecies richness, where subspecies overlap in range and/or hybridize, are concentrated along the course of the Amazon River, with the eastern Andes slopes and foothills relatively depauperate in terms of local intraspecific phenotypic diversity. Spatial gradients in heliconiine species richness in the Neotropics are consistent with the hypothesis that species richness gradients are driven at least in part by variation in speciation and/or extinction rates, resulting in observed gradients in mean phylogenetic branch length, rather than via evolutionary age or niche conservatism alone. These data, coupled with individual case studies of recently evolved *Heliconius* species, suggest that the radiation of heliconiine butterflies occurred predominantly on the eastern slopes of the Andes in Colombia, Ecuador and Peru and in the upper/middle Amazon basin.

Introduction

Understanding the processes responsible for spatial variation in species richness is a central goal in ecology and evolution (Rosenzweig 1995, Ricklefs and Miller 1999, Gaston 2000, Hawkins et al. 2003). It is also a vital prerequisite to conservation of the earth's living resources in the face of rapid environmental change (Myers et al. 2000, Lamoreux et al. 2006). However, despite broad agreement that, in general, community species richness increases from the poles to the tropics, there remain many conflicting hypotheses to explain this pattern (Willig et al. 2003, Mittelbach et al. 2007). These hypotheses are often grouped into three major classes: null, ecological or evolutionary. Proponents of the geometric constraints model argue that a tropical peak in species richness can result from stochastic placement of species ranges within a bounded domain (Colwell and Hurtt 1994, Rosenzweig 1995, Willig and Lyons 1998, Colwell and Lees 2000). Such models assume nothing about the ecology or evolution of species, such as the importance of competition, or variation in rates of speciation or extinction. Ecological models suggest that species richness is a result of the number of niches available, which are in turn affected by primary productivity and ultimately climate. However, whether or not communities present at different latitudes are at regional dynamic species richness equilibria is questionable (Rees et al. 2001). By contrast, evolutionary models suggest that tropical richness is a result of higher diversification rates or longer time for diversification in more tropical regions; in these models, community species richness does not need to be at equilibrium (Mittelbach et al. 2007). Ecological and evolutionary explanations need not be mutually exclusive; for instance, it is possible that ecological limits lead to a negative feedback of diversity on speciation rates (Rabosky 2009).

Among ecological and evolutionary hypotheses for high diversity of species in the tropics, perhaps the oldest is "the evolutionary age" hypothesis: that tropical regions have been more stable over geological time, permitting “a comparatively continuous and unchecked development of organic forms” (Wallace, 1878: 123; Fischer, 1960; Wiens *et al.*, 2009). A related hypothesis is "tropical niche conservatism." It states that most taxa originate at tropical latitudes and rarely colonize higher latitudes because of phylogenetic niche conservatism (Wallace 1876, Wiens and Donoghue 2004, Hawkins et al. 2005, 2006, 2007, Wiens et al. 2009). Neither evolutionary age nor tropical conservatism hypotheses require variation in rates of speciation or extinction to drive species richness gradients (Wiens et al. 2009); instead, more species are assumed to accumulate in more tropical regions because of longer favourable periods for diversification. A clear alternative is that diversification rates are higher in more tropical regions, either because of higher speciation rates (e.g., Cardillo *et al.*, 2005), or lower extinction rates (e.g., Wallace, 1878; Wiens, 2007).

Here, I present detailed range maps for a diverse and well-studied Neotropical group, the heliconiine butterflies (Lepidoptera: Nymphalidae: subtribe Heliconiina). I combine these data with molecular phylogenetic information and use them to test evolutionary hypotheses for species richness gradients. The evolutionary age and tropical conservatism hypotheses posit that species-rich tropical clades originated at low latitudes, and that richness gradients are then a product of “time for speciation” and infrequent dispersal to higher latitudes. Species in less diverse areas should therefore be younger or at the most equal in age to those occurring in more diverse areas (e.g., Hawkins & DeVries (2009). Finding that species in less diverse areas tend to be older would cast doubt on evolutionary age or niche conservatism as the sole explanations for the species richness gradient, and imply that speciation and/or

extinction must play a role. I test this hypothesis using the terminal branch length of a relaxed clock molecular phylogeny, which will provide an overestimate of species age due to coalescence of gene trees typically predating speciation and extinction pruning tips from the tree (Edwards and Beerli 2000). Assuming no geographic variation in speciation and/or extinction rates, the evolutionary age and niche conservatism hypotheses both predict that across assemblages mean terminal branch length should either be positively related or unrelated to species richness. If differences in evolutionary age or niche constraints operated relatively recently and affected extant species, then their influence on terminal branch lengths should still be apparent and branch lengths will be positively related to species richness. However, if the hypotheses were relevant only before extant lineages appeared, then recent speciation and extinction events could erase historical phylogenetic signal, resulting in no association between terminal branch length and species richness. In contrast to these patterns, higher speciation rates or lower extinction rates both result in assemblages composed of species with shorter mean terminal branch lengths, resulting in a negative relationship between mean branch length and species richness.

In addition to a global tendency for tropical areas to contain more species than temperate areas, there is substantial variation among different tropical regions, with the Neotropical region thought to contain more species of plants, amphibians, birds and mammals than either the African or Asian tropics (Gaston and Hudson 1994, Thomas 1999). While many hypotheses consider lowland rainforests as the origin for Neotropical biodiversity (Haffer 1969, 2008), recent studies have shifted the focus onto the Andes as a source of speciation. The diverse topography of the Andean region may present more opportunities for allopatric speciation than the lowlands and may also provide suitable conditions for ecological speciation (Chapman 1917), with

a recent study suggesting that this could be responsible for the unusually high butterfly species richness of the Neotropics (Elias et al. 2009). Fjeldså (1994; Fjeldså *et al.*, 1999) suggested on the basis of bird studies that Andean valleys and plateaus which were buffered from climatic fluctuations functioned as a "species pump" for South America, with new narrowly endemic species arising continuously over time. In contrast, the lowlands were seen as unstable climatically, characterised by frequent extinctions, and appeared to be a "museum" for widespread, older lineages. To address these questions with the butterfly dataset, I map the distributions of subspecies and intersubspecific hybrids in addition to species richness and phylogenetic branch length. This allows identification of areas of unusually high polymorphism and subspecies diversity. If subspecies represent incipient species (Mayr 1942), areas where subspecies richness is high and hybridisation between subspecies is common may correspond to areas where speciation is initiated, or to "suture zones" where evolving taxa meet and form hybrid zones (Remington 1968).

The heliconiine butterflies are a colourful and diverse neotropical group well known for their participation in Müllerian mimicry rings. Heliconiines have served extensively as subjects for studies in evolution, ecology and genetics and are among the best studied insects of no commercial importance (Brown, 1981; Turner, 1981). Heliconiine species are normally recognized by differences in wing colour pattern and few intermediates in sympatry, but also by means of genitalic morphology and in some cases genetic data (e.g., Jiggins et al. 1996, Brower 1996, Jiggins and Davies 1998, Giraldo et al. 2008). Subspecies are recognized by geographical differences in wing colour pattern and abundant hybrid genotypes in contact zones (Holzinger and Holzinger 1994). Many subspecies of heliconiines do not fit the definition of subspecies as geographically separated phenotypes (Brown 1979, Turner 1981, Mallet

2001). Rather, in a number of regions, multiple phenotypes co-occur, likely maintained by mimicry with alternative models (Joron et al. 1999). Previous work on the biogeography of heliconiines demonstrated broad patterns of species richness and subspecies endemism (Emsley 1963, Brown 1979, Holzinger and Holzinger 1994). Since these studies, however, many new distribution data have become available and there have been significant revisions to heliconiine taxonomy. In addition, new GIS technology has enabled distributions to be mapped at a much finer resolution using data that are easily retrievable and verifiable, resulting in range maps for 434 subspecies in addition to 70 species. These provide the most detailed georeferenced range maps for any Neotropical insect group to date and a useful digital source of data for others interested in heliconiines or insect biogeography in general.

Methods

Point locality data

I compiled a database of point locality records for the Heliconiina, using a modified version of the most recent taxonomic checklist (Lamas 2004; table A.1.1 in appendix 1). The principal sources for data were museum collections (primarily those in two of the world's largest collections, the Natural History Museum in London (NHM) and the Florida Museum of Natural History in Gainesville (FLMNH)), research databases and the scientific literature. The data sources used are summarised in Table 2.1. Point locality records in the database refer to individual specimens when sourced from collections and research databases, but to localities where the species occurs when sourced from literature. Older museum specimens are often labelled with very generalised localities (e.g. "Nouvelle Grenade"). To remove imprecise locality data from the analysis, I obtained a crude measure of precision when georeferencing a

locality (a national park, for instance) based on the extent of the area to which the name refers. I measured the maximum radius from the inferred central coordinates of the locality to the edge of the area and included these as "point" localities only where precision was < 40km. Museum specimens are also often mislabelled, especially if collected commercially, where little importance may be placed on precise localities, or where the locality labels are deliberately misleading (Emsley 1963). I therefore excluded any localities that appeared to be clearly erroneous, i.e. where a data label of low reliability indicated a locality point significantly outside the otherwise known distribution of the species. When unsure of the reliability of data, I consulted with experts with specific knowledge about the particular sampling region.

If a specimen was identified as an interspecific or intraspecific hybrid (displaying a mix of colour pattern elements from other taxa), I treated it as a locality record for both putative progenitors. All ten putative species of *Philaethria* were excluded (except for maps of sampling effort) due to taxonomic uncertainty as to species limits: recently, a number of cryptic species have been described (Constantino and Salazar 2010). *Laparus doris* (Linnaeus) also created problems for mapping because it is highly polymorphic and shows wide clinal variation (Mallet 1999), which has resulted in inconsistent application of subspecific nomenclature to specimens in major collections. I therefore excluded *L. doris* from analysis of subspecies distributions.

Distribution maps

All GIS-based work was carried out in ArcMap 9.3 (ESRI, Redlands, CA), unless otherwise stated. I chose not to apply a species distribution modelling approach in the present study as many heliconiine subspecies (and some species) are known from very few (< 10) localities. Instead, I explored two simpler approaches for converting point

locality data into predictions of species' distributions. As a first step, I used a minimum convex polygon (or convex hull) method, which estimates a species range as the smallest polygon encompassing all of the data subject to the constraint that no internal angle can be greater than 180º (Sheth et al. 2008). However, the method gives an unrealistic extent of occurrence when a species range includes a real concave boundary. This can result in the extent of occurrence map including large areas of inhospitable habitat where the focal taxon is known to be absent. To correct for this, I used a related method, α-convex hulls, which allows for convex margins and gaps within a species ranges (Edelsbrunner et al. 1983, Burgman and Fox 2003). A difficulty with the α-convex hull approach lies in deciding what area should constitute a real gap in a species range. This is particularly problematic in poorly sampled areas such as the Amazon. I found that the maps that most consistently met my subjective *a priori* expectations of species and subspecies ranges were made by applying α-convex hulls with α set to 1400km, which was the smallest value that resulted in no discontinuous ranges for any species. I obtained this value by increasing the value of α incrementally until all species ranges formed single continuous polygons. Thus using the α-convex hull approach returns similar results to the minimum convex polygon approach, but allows for ranges to have convex margins at broader scales. I created α-convex hulls in the R package alphahull (Pateiro-López and Rodríguez-Casal 2010) using point locality data projected to a Lambert Cylindrical Equal Area projection.

Many of the resulting distributions still contained areas where a particular species was thought highly unlikely to occur, so clipping of the resultant range maps was required. I clipped the polygons to coastlines and the altitudinal ranges of the species using a 30 arc-second altitudinal grid obtained from WorldClim (www.worldclim.org). When the

polygon for a species indicated presence on islands, I only included the islands with known records for the species. Altitudinal clipping was based on published information (Brown and Mielke 1972, Brown 1979, DeVries 1987) and consultation with experts. The elevational boundaries used for clipping are shown in Appendix 1 (Table A.1.2). I also clipped polygons to exclude well-sampled areas where I could be confident that an absence of records is not an artefact, for example in cases where areas west of the Andes were inferred to contain otherwise Amazonian species.

Grid-cell based analyses

Species richness, subspecies richness and sampling effort were mapped using a 50km x 50 km grid and Lambert Cylindrical Equal Area projection to ensure equal area sampling. Sampling effort was estimated as the number of geographical records in each grid cell. Species and subspecies richness were estimated as the count of species or subspecies ranges that overlap each grid cell. I also present a second map of species richness which was created by merging the subspecies ranges for each species, and using the resulting distributions to map species richness. The map of subspecies richness therefore differs only from the second map of species richness in that subspecies within species are superimposed due to polymorphism in traits considered diagnostic for subspecies, allowing direct comparisons between species and subspecies richness patterns. Finally, to identify areas with unusually high numbers of subspecies (once the number of species present is taken into account), I mapped the average number of subspecies per species in grid cells.

Molecular phylogenetic information comprised mtDNA divergence-based branch lengths estimated using a relaxed clock method with a multilocus sequence-based phylogeny of the Heliconiina (methods described in detail elsewhere, see Beltrán et

al. (2007) and Mallet et al. (2007)). I mapped the mean terminal branch length of assemblages as described by 100km x 100km equal area grid cells. To test whether mean terminal branch length is related to species richness I used Pearson's correlation. Given the problem of spatial autocorrelation, I used the program SAM (Spatial Analysis in Macroecology) (Rangel et al. 2010) to calculate the geographically effective degrees of freedom and correct p-values (Dutilleul et al. 1993). The degree to which two cells/assemblages have similar mean values will be influenced by the number of species that they share. For instance, any two cells that share all the same species must have the same mean terminal branch length. Correcting for non-independence among cells due to spatial autocorrelation should partially deal with this problem, though some non-independence may remain if the degree to which cells share species does not decline over space at a constant rate.

In order to identify possible intraspecific suture zones (Remington 1968), I selected all specimens that had been identified either as subspecies hybrids or as taxa listed by Lamas (2004) as hybrids. I then calculated the proportion of species with hybridising subspecies in equal area grid cells of 100km x 100km: potential subspecific suture zones can be therefore be recognised as grid cells where a high proportion of species have hybridising subspecies. While I use the term "suture zone" here, a high proportion of subspecific hybrids may arise from on-going divergence as well as the meeting of already diverged populations (Dasmahapatra et al. 2010). To account for biases induced by small sample sizes, I only included grid cells with ≥ 20 specimen records. To investigate whether sampling effort affects the proportion of hybrid specimens, I used a general linear model (GLM) treating proportion of hybridising species as a response and with binomial errors and log sample size as a predictor. I back-transformed the residual variation from the logit scale to proportions and

mapped it. I then compared the map of residual variation with the map of proportions of hybridising species.

Results

Data

I collected a total of 58,059 geographical records for 70 species and 434 subspecies of heliconiines, with information from 10,046 point localities. I excluded 3,901 records of dubious authenticity or imprecise locality information. The database has been made available on-line (www.ucl.ac.uk/taxome/neil_rosser), enabling users to download data and plot species and subspecies distributions.

For those localities where a measure of precision could be made, 85% of locality names referred to an area that extended < 40km from the central coordinates, and 74% to an area extending < 10km from the central coordinates. Specimen collection date and precision varied among collections. For example, comparing the two collections which provided the largest number of records, specimens in the Florida Museum of Natural History (FLMNH) had a mean collection date of 1984 in contrast to 1921 in The Natural History Museum (NHM), while 87% (FLMNH) vs. 79% (NHM) of specimens could be assigned to the nearest 40km, and 77% (FLMNH) vs. 68% (NHM) could be assigned to the nearest 10km.

Table 2.1. Summary of data sources

Source	Website	Number of records
The Florida Museum of Natural History	http://www.flmnh.ufl.edu	20881
The Natural History Museum (London)	http://www.nhm.ac.uk	8277
Brown (1979)		6079
Tropical Andean Butterfly Diversity Project	http://www.mariposasandinas.org	4078
CONABIO, Mexico	http://www.conabio.gob.mx	3433
Museo de Historia Natural, Lima, Peru	http://museohn.unmsm.edu.pe	3067
C. Jiggins research database	http://*Heliconius*.zoo.cam.ac.uk	1780
INBio, Costa Rica	http://www.inbio.ac.cr	1682
K. Willmott and J. Hall research database	http://www.flmnh.ufl.edu/butterflies/neotropica	1479
J. Mallet research database	http://abacus.gene.ucl.ac.uk/jim	1203
"Butterflies of Colombia", LeCrom (in prep.)		1179
Butterflies and Moths of North America	http://www.butterfliesandmoths.org	1118
A. Brower research database	http://frank.mtsu.edu/~abrower	960
speciesLink	http://splink.cria.org.br	618
Other sources (published studies, websites etc).		2228
Total:		**58062**

Sampling effort

A map of sampling effort is shown in Figure 2.1. Tropical Mexico and Central America are generally well sampled, but there are few data from Nicaragua and Honduras. The dearth of records from the Caribbean islands is probably not a good indication of overall museum sampling effort, as few heliconiines occur there. In South America, Andean regions are for the most part well sampled. In comparison, very few collections are available from across vast areas of Amazonia and the Guiana shield. Notable exceptions include various sites along the main course of the Amazon River, the Ariquemes area in Rondônia, Brazil (intensively collected by K. Brown, G. Austin and others in the 1970s and 1980s), coastal French Guiana and northern Guyana (the latter two well represented in the NHM collections). Eastern Colombia has been poorly sampled, but it is probable that the lack of records from the Llanos of Colombia and Venezuela is in part due to the scarcity of heliconiines in such ecosystems (where they are confined to gallery forest). Similarly, it is likely that the lack of samples from Western Peru, Chile, and the interior of north-eastern Brazil is due as much to the fact that few heliconiines occur there as it is to low collecting effort.

Species and subspecies distributions

Distribution maps for species and subspecies are presented in appendix 1. Figures 2.2A and 2.2B show example maps for a lowland species (*Heliconius antiochus* (Linnaeus)) and a mid-elevation species (*Heliconius telesiphe* (Doubleday)).

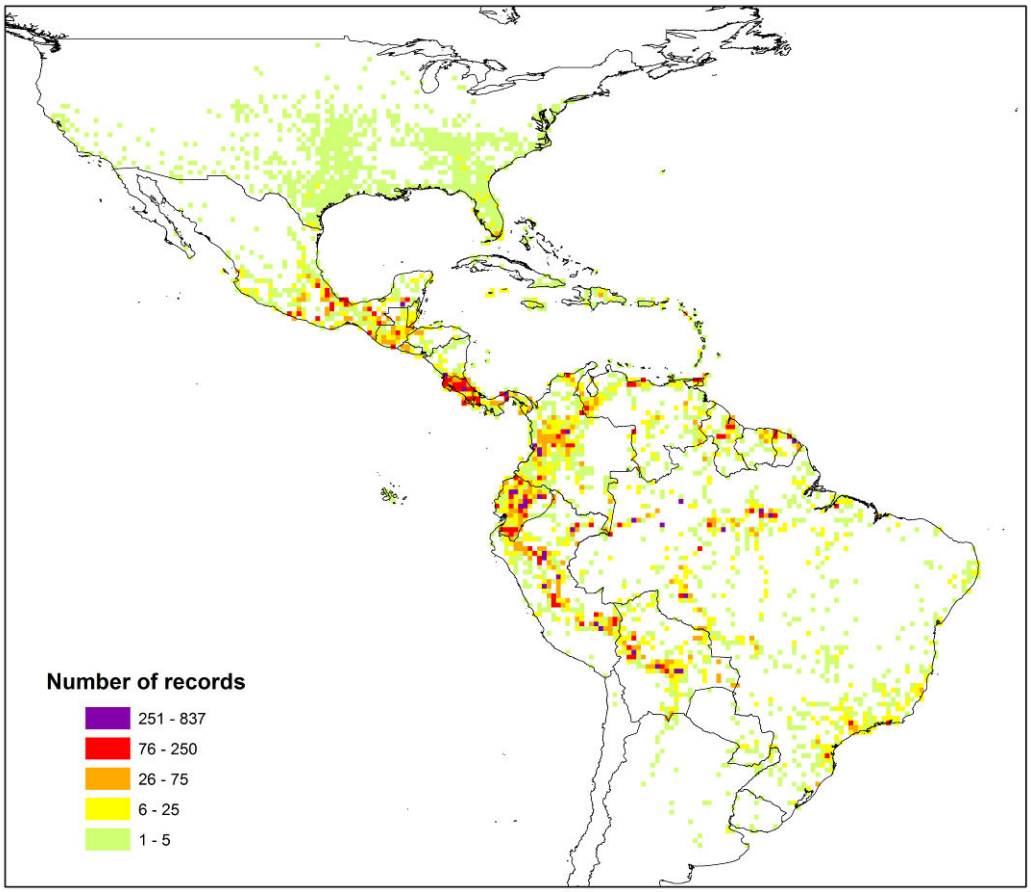
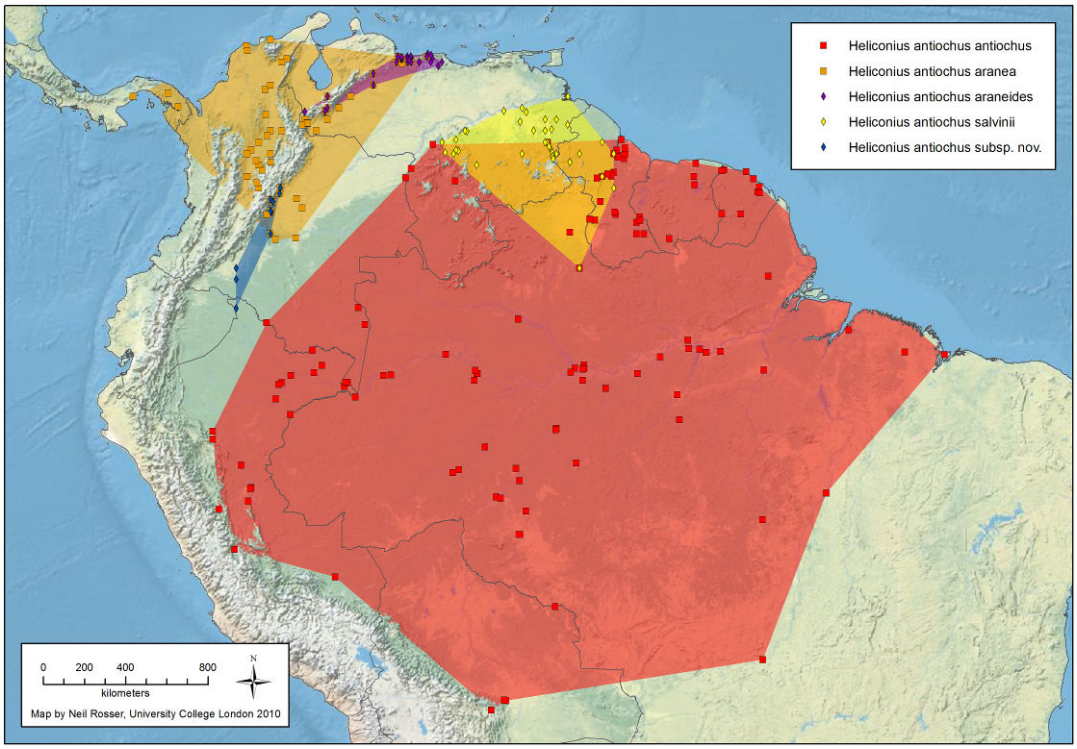


Figure 2.1. Sampling effort mapped in 50km x 50km grid cells



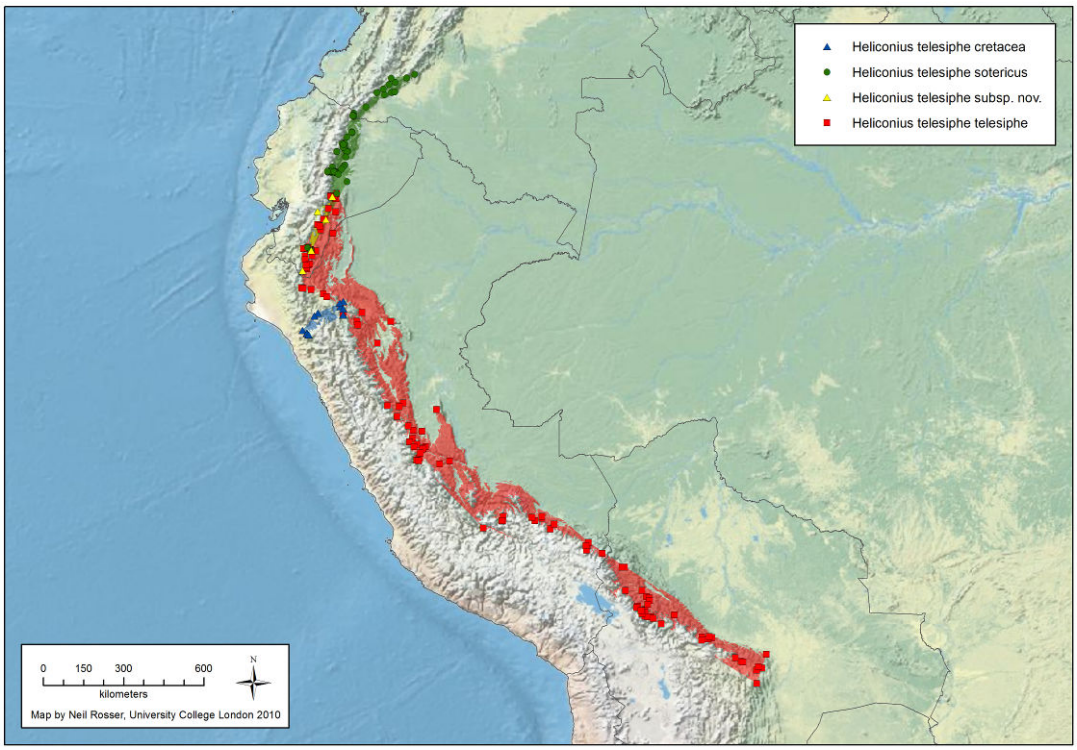


Figure 2.2. (A) Range maps for the subspecies of *Heliconius antiochus* and (B) *Heliconius telesiphe*.

Patterns of species richness

Species richness of heliconiines is highest in the Amazon basin and adjacent slopes of the Andes, the Guiana shield, central and western Colombia and north-west Ecuador (Figure 2.3A). Across this area the number of species in 50km grid cells rarely dips below 25, with the Andean – Amazonian ecotone of Colombia, Ecuador and Peru comprising the most speciose region of all (up to 40 species / 50km x 50km grid square in the vicinity of Mocoa, Putumayo). The Manaus area in the central Amazon forms another hotspot, but here richness does not exceed 31 species / 50km x 50km grid square. The Llanos of Colombia and Venezuela have low species richness compared to surrounding areas. Central America and southern Mexico are relatively species rich (up to 24 species / 50km x 50km grid square in Panama), but only four species have colonised the Caribbean. Central and eastern Brazil appear depauperate and the Atlantic rainforests of south-east Brazil do not show up as a richness hotspot for heliconiines. Very few heliconiine species occur on the Pacific coast of South America south of Ecuador, where rainfall is extremely low. In order to make direct comparisons between maps of species and subspecies richness, I present a second map of species richness based on the merged subspecies ranges for each species, rather than the overall species' ranges (Figure 2.3B). Note that in this map species richness counts tend to be lower because the constituent species range estimates are typically smaller.

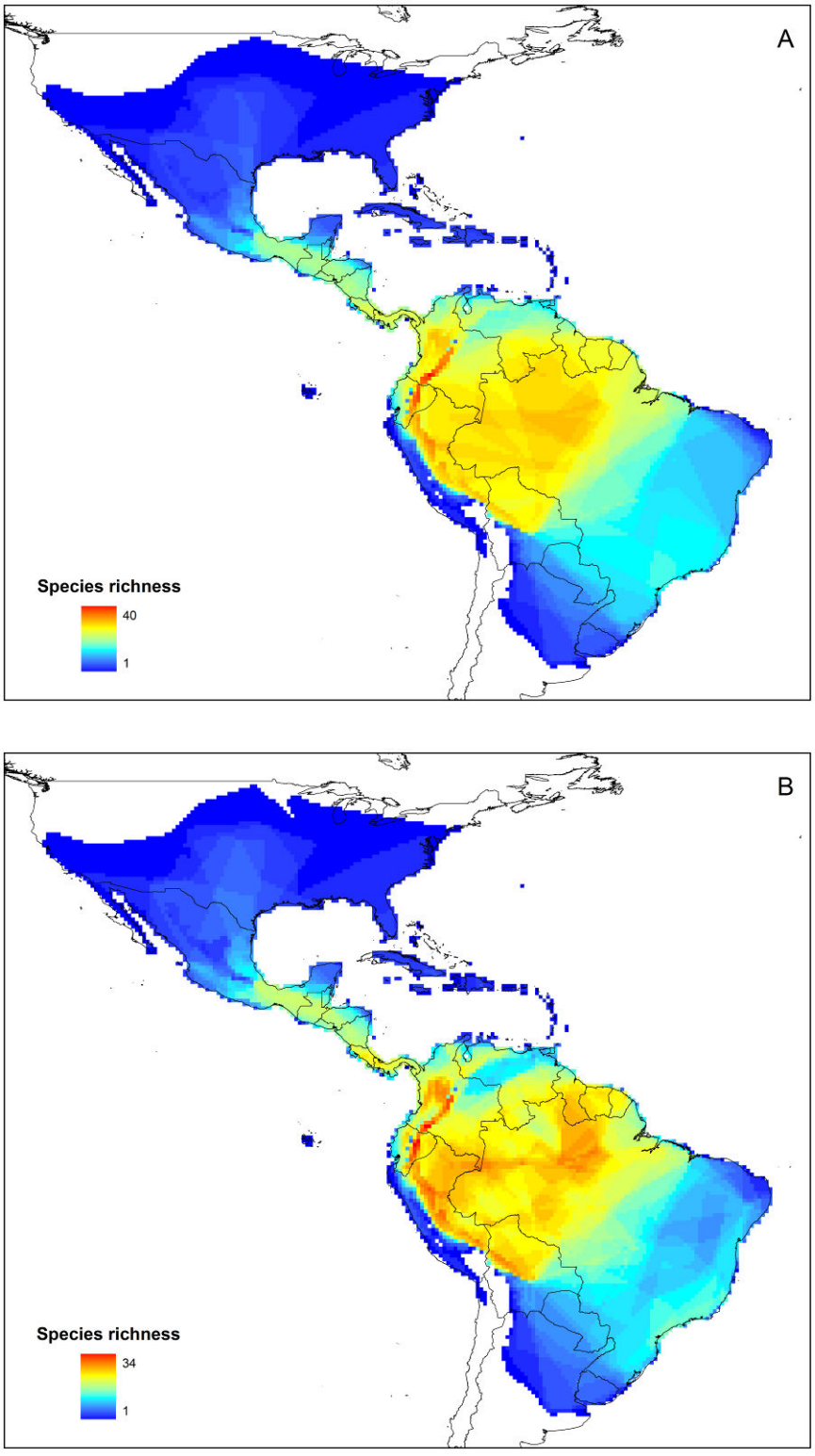


Figure 2.3. (A) Species richness mapped using species polygons, (B) species richness based on the union of subspecies polygons. 50km x 50km grid cells.

Figure 2.4 maps the average terminal branch lengths (a proxy for species age) of species in equal area grid cells. Interestingly, there is a weak tendency for terminal branch lengths tend to be short in the regions of highest species diversity, especially on the eastern slopes of the Andes, and longer in the less diverse regions. However, there are a few areas on the edge of the distribution where a young average age is concentrated on very low diversity regions which may be ascribable to an increase in the variance of the mean as species richness declines (as in a funnel plot, Figure 2.5). Species richness is negatively related to species age (Figure 2.5), but the relation is marginally insignificant when spatial autocorrelation is taken into account (Pearson's r = -0.704, corrected degrees of freedom = 5.751, corrected probability = 0.057).

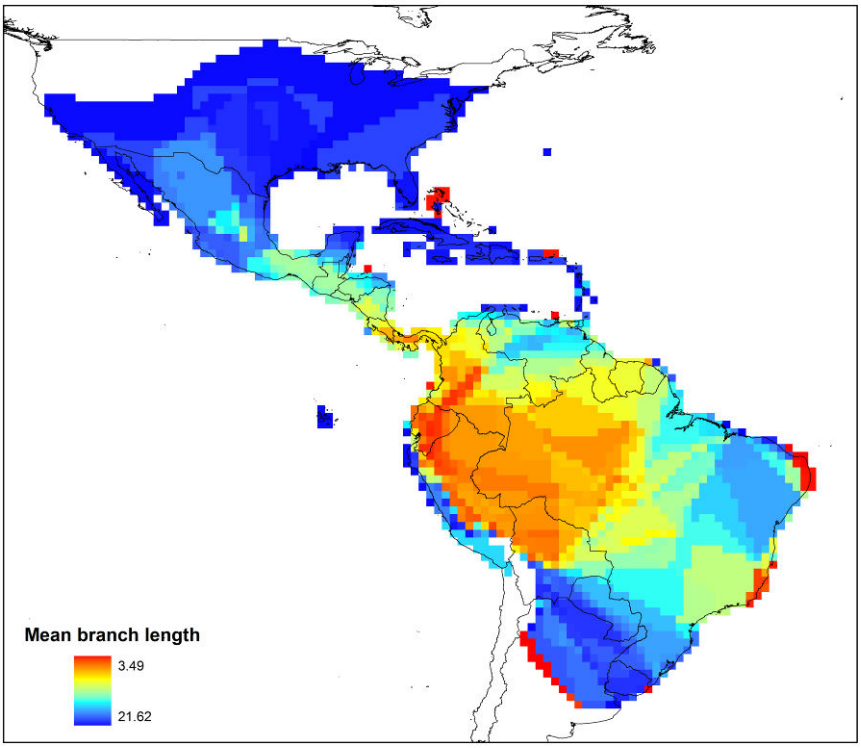


Figure 2.4. Mean mtDNA terminal branch length of species mapped in 100km x 100km grid cells.

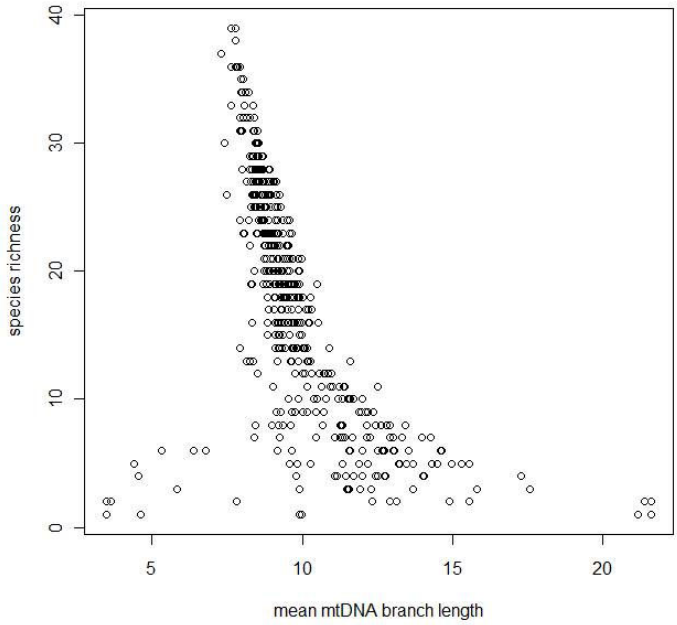


Figure 2.5. Plot of mean mtDNA terminal branch length against species richness in 100km x 100km grid cells. Note that these data points are not statistically independent due to nearby cells often sharing the same species.

Subspecies richness and suture zones

Patterns of subspecies richness (Figure 2.6A) show several differences from those of species richness. It should be emphasised that this map of richness can differ from that in Figure 2.3B because multiple subspecies of a single species may occur within a single cell when the cell is located at a subspecies range margin, or because subspecies are sympatric due to polymorphism in traits considered diagnostic for subspecies. Notably, regions near the Amazon River appear by far the richest area in terms of subspecies with as many as 63 subspecies present in a 50km grid square at Manaus, Brazil. In contrast, richness in the Amazon-draining foothills of the eastern Andes of Ecuador and southern Colombia does not exceed 52 subspecies in a 50km grid cell, even though species richness is higher there. Figure 2.6B maps the average number of subspecies per species. The contours of this map are similar to those of

subspecies richness (Figure 2.6A), showing not only that areas surrounding the Amazon river and its tributaries have the most subspecies per grid cell but that these areas are still especially subspecies-rich once species richness is taken into account. In addition, certain patterns emerge that are not apparent in the subspecies richness map. Most strikingly, the eastern Andes of Colombia, Ecuador and Peru, the Magdalena Valley in Colombia, Western Ecuador and Central America all have relatively few subspecies per grid cell given the number of species that occur there.

The possibility that the conflicting patterns of species and subspecies richness are to some extent the result of a sampling artefact cannot completely be discounted. The Amazon basin is much less well sampled relative to the Andes, and consequently Andean foothill taxa may be more likely to be recognised as species. Indeed, recent ecological and molecular phylogenetic studies of Andean taxa have resulted in taxa formerly ranked as subspecies being elevated to species status on the grounds of bimodality in hybrid zones, as well as the discovery of cryptic species (Jiggins et al. 1996, Brower 1996, Jiggins and Davies 1998, Giraldo et al. 2008, Arias et al. 2008, Mallet 2009). However, these recent taxonomic changes in themselves should not substantially affect the conclusions drawn here, because reclassifying the species concerned as subspecies would at most reduce species richness in the Andes by 2 per grid cell, and likewise elevate subspecies richness by 2 per grid cell. Nonetheless, the possibility remains that future study in the Amazon could show species richness to be underestimated, and subspecies richness to be overestimated.

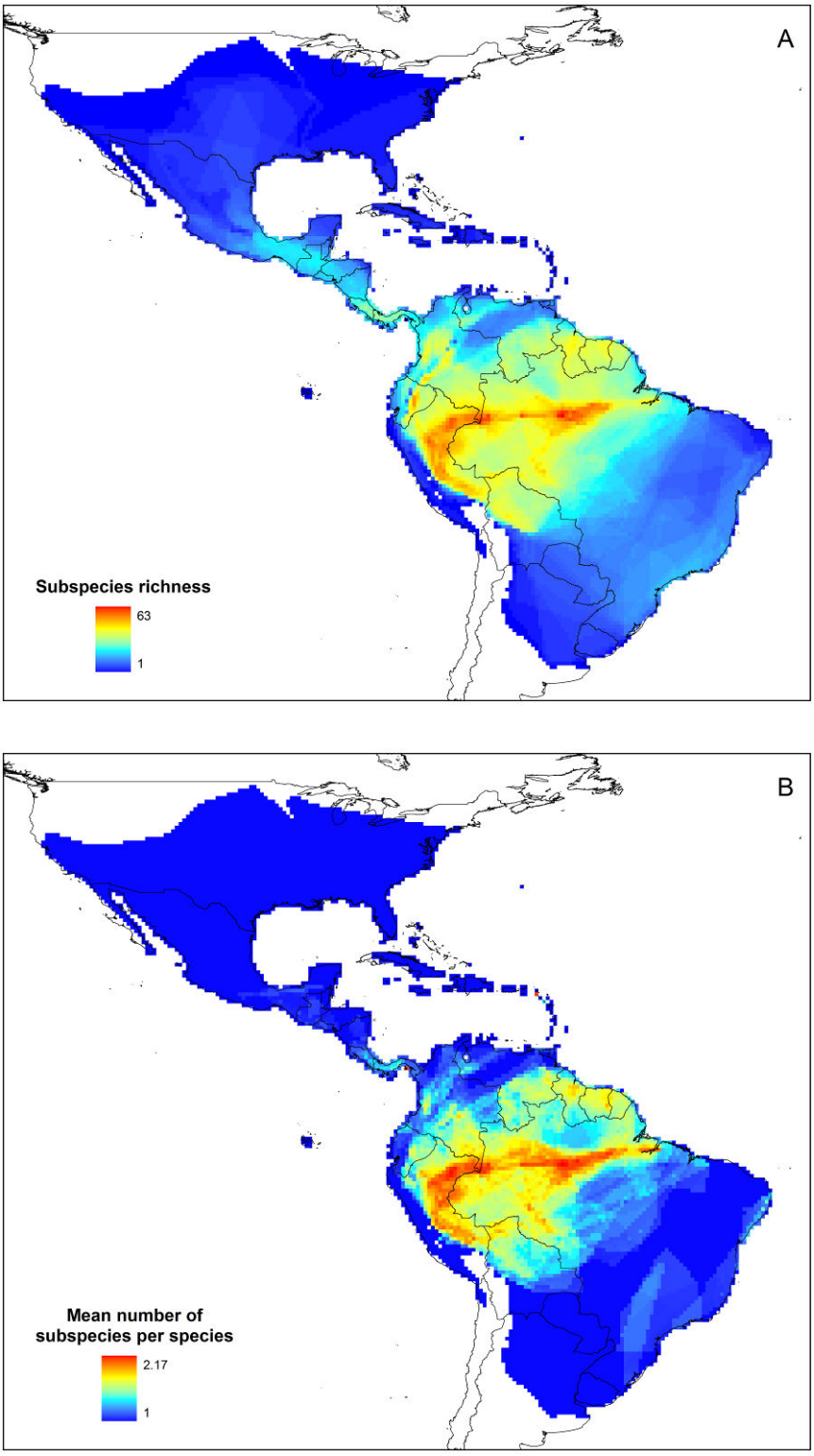


Figure 2.6. Areas of high subspecies turnover. (A) subspecies richness and (B) mean number of subspecies per species in 50km x 50km grid cells.

The proportions of species with hybridising subspecies were mapped in 100km x 100km equal area grid cells to further identify possible suture zones (Figure 2.7A). Most of the cells with high proportions of hybridising species are found along the course of the Amazon River to the foothills on the Andes in Peru. I used a GLM with binomial family errors to investigate the extent to which sampling effort affects this analysis. I mapped the residual variation from this model (Figure 2.7B); cells with high values correspond well to those in Figure 2.7A, suggesting the geographic patterns cannot be explained solely by sampling artefacts.

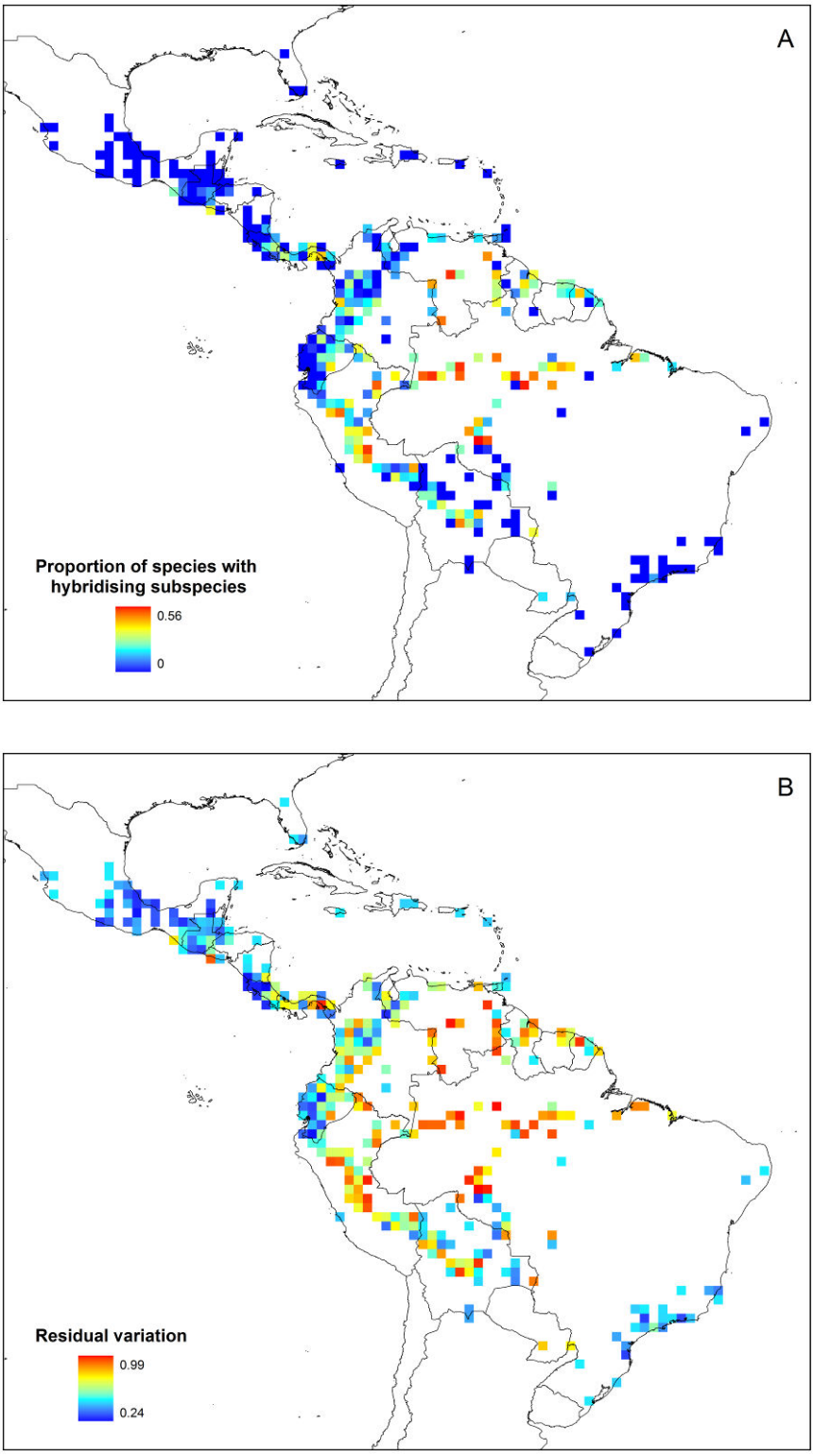


Figure 2.7. (A) Proportion of species with hybridising subspecies mapped in 100km x 100km grid cells. To account for biases induced by small sample sizes, we only included grid cells with ≥ 20 specimen records. (B) Residual variation from general linear model treating proportion of hybridising species as a response and with binomial family error structure and log sample size as a predictor, backtransformed to proportions.

Discussion

Geographic origin of heliconiine species richness

At broad scales, these results confirm that the widespread latitudinal gradient in species richness also occurs in Heliconiina, not only between temperate and tropical regions, but also, importantly, within the tropical region itself (Figure 2.3A,B). The average terminal phylogenetic branch length of species (Figure 2.4) tends to be shorter in the eastern Andes and Amazon basin, with terminal branch lengths becoming progressively longer on average through Central America and southeastern Brazil and then into more temperate regions (a few peripheral regions with young average age are due to stochastic variation when the numbers of species tested are very low). Overall, the average age of species appears to be negatively related with species richness (Figures 2.4, 2.5), with the richest areas inhabited by predominantly young species. This pattern is consistent, at a local scale, with variation in speciation and/or extinction rates driving the negative relationship between species richness and latitude (e.g., Wright *et al.*, 2006; Allen *et al.*, 2006; Allen & Gillooly, 2006; Jablonski *et al.*, 2006) and argues against evolutionary age or niche conservatism as the sole explanations.

The results are consistent with a recent study of New World limenitidine nymphalid butterflies (Mullen et al. 2011), which also found evidence for higher diversification rates, rather than evolutionary age, driving the marked asymmetry in species richness between tropical *Adelpha* and temperate *Limenitis*. However, in another recent study of North American butterflies, Hawkins & DeVries (2009) found a pattern of increasing "mean root distance" (MRD, an ordinal measure of the mean number of nodes between the root of the tree and the tips) with increasing latitude, which they

interpreted as evidence for mainly recent evolution of butterflies into more temperate habitats. This, it was argued, was consistent with tropical conservatism as an explanation for low temperate species richness. The latter results might appear to contradict the present findings of younger, not older, species in the more diverse parts of the central tropics. However, their clade youth measure was of subfamily "mean root distance", and there is no necessary reason to expect a relationship with age at the level of species. Furthermore, their data tended to exclude the most diverse, central tropical regions. Nevertheless, the spatial patterns of MRD found by Hawkins & DeVries (2009) are certainly striking. These patterns may instead result from evolution of cold tolerance and colonization of relatively recent temperate biomes, in particular grasslands, in only a few clades that contain the greatest potential for rapid adaptation. Such clades are also likely to be those that are diversifying most rapidly (e.g., Nymphalidae, Lycaenidae) and therefore have a higher MRD. An additional requirement of the tropical conservatism hypothesis, namely that most clades have a tropical origin (Hawkins & DeVries 2009), requires evaluation in most neotropical taxa. However, there is considerable evidence in favour of the opposite scenario, namely that most neotropical clades evolved from temperate or montane ancestors. For example, the ancestor of *Adelpha* was almost certainly a temperate or montane species, since Limenitidini phylogeny supports an Old World origin of the group with subsequent colonization of the New World via the Bering Strait in the middle Miocene (12.5-15Ma) (Mullen et al. 2011). Similarly, northern routes are inferred for the diverse neotropical clades Phyciodina (Wahlberg, 2006; Wahlberg & Freitas, 2007), Euptychiina (Peña and Wahlberg 2008), and Aporiina (Braby et al. 2007), and a southern route for *Euryades+Parides* (Braby et al. 2005). Clearly, phylogenetic hypotheses for multiple groups are needed to examine the generality of the various

evolutionary hypotheses proposed to explain the latitudinal gradient in New World butterfly species richness.

Within the neotropical region, heliconiine species richness clearly peaks along the eastern slopes of the Andes, where richness is highest for species of all ages. This pattern is common in other neotropical groups, including cicindelid beetles (Pearson and Carroll 2001), birds (Orme et al. 2005) and mammals (Willig et al. 2003), and corroborates results from other groups of butterflies, including several aposematic groups (Brown 1982) and limenitidines (Willmott 2003, Mullen et al. 2011). Partly, this pattern can be explained by high community species richness (α diversity), and partly by high rates of species turnover across the Andean elevational gradient (β diversity), with a mix of montane and lowland species occupying grid cells in the eastern Andean foothills. For example, the large but almost entirely lowland butterfly genus *Theope* shows little difference in species richness across the Amazon basin, probably because it lacks montane species to inflate grid cell totals in the Andean foothills (Hall 1999).

Whatever the cause of high species richness in the east Andean foothills, these data support an important role of the Andes in the evolution of heliconiines, a view bolstered by case studies of Andean heliconiine taxa close to the species boundary (Jiggins et al. 1996, Brower 1996, Jiggins and Davies 1998, Mavárez et al. 2006, Giraldo et al. 2008, Arias et al. 2008). The radiation of the most diverse heliconiine genera (which comprise about 85% of the species) has taken place during the last 18 million years (Wahlberg et al. 2009), coinciding with the major period of Andean uplift (Gregory-Wodzicki 2000), and notably species terminal branch lengths are on average particularly short in the eastern Andes. Nevertheless, species richness in the

Amazon basin is also high and the average terminal branch length are also short, suggesting that the lowlands have produced much of the heliconiine diversity.

In contrast, "subspecies richness", reflecting intraspecific polymorphism of colour pattern traits, is highest along the course of the Amazon River, with the Eastern Andes relatively depauperate (Figure 2.6A,B). The map of the proportions of species with hybridising subspecies (Figure 2.7A,B) suggests the existence of a suture zone (Remington 1968) which corroborates this pattern of phenotypic diversity; many grid cells with high proportions are closely associated with the area of maximum subspecies overlap and richness along the Amazon River (Figure 2.6A). Many subspecies have their range limits near the Amazon (see appendix 1), and the region may form a discontinuity between faunas. A partial barrier effect of major rivers is well known from birds and primates (Capparella 1990, Ayres and Clutton-Brock 1992, Burney and Brumfield 2009), but the distribution maps show that species and subspecies ranges often span even the widest rivers. Thus while even the Amazon does not form an impregnable barrier for all heliconiines, it may serve to reduce gene flow sufficiently to slow the spread of novel phenotypes on either side leading to the formation of a suture zone.

What might explain the apparent discrepancy between areas with high species richness and those with high subspecies richness and levels of inter-subspecific hybridisation? A likely explanation is that the majority of lowland subspecies do not represent incipient species. While many species probably go through a stage of being subspecies, the formation of locally adapted populations does not necessarily result in speciation (Butlin et al. 2008). Although speciation in *Heliconius* is frequently associated with a switch in mimetic colour pattern (Jiggins et al. 2001, Mallet 2009),

most Amazonian subspecies represent relatively minor variations within broad mimicry rings, and high rates of gene flow may prohibit further divergence among local polymorphs (e.g., Joron and Mallet 1998). In contrast, subspecies occurring close to the Andean cordillera often have more divergent colour patterns, and speciation may more readily be completed here. This could be due to the Andes providing more barriers to gene flow, or because the more spatially heterogeneous environmental conditions (particularly across elevations) present greater opportunities for ecological speciation (Elias et al. 2009), or a combination of the two.

Another possibility that might explain the differences in subspecies and species diversity patterns is that subspecies in the Andes are older, allowing more time for speciation, and so a greater preponderance of young species and fewer remaining subspecies. It is hard to estimate the ages of subspecies; in fact the concept may often be meaningless if different parts of the genome have very different histories. However, in both *Heliconius erato* (Linnaeus) and its unrelated mimic *Heliconius melpomene* (Linnaeus), there is now molecular evidence from the red colour pattern and Müllerian mimicry locus, *optix*, that the "rayed" Amazonian colour patterns are very closely related to each other in comparison with the peripheral, Andean "postman" subspecies with unrayed colour patterns. This suggests that rayed mimetic colour patterns are a recent Amazonian innovation that has spread out, confining older, extra-Amazonian mimicry colour patterns to relictual, and in some cases disjunct populations in the periphery of the Amazon (Hines et al. 2011). This centrifugal spread hypothesis was predicted for *Heliconius* warning colour originally on the basis of disjunct colour patterns in the periphery of the Amazon, and because it was consistent with other evidence for a "shifting balance" mode of warning pattern diversification (Mallet 1993, 2010, Turner and Mallet 1996). Rapid evolution and

turnover of new colour patterns in the Amazon, rapid spread to the periphery, followed by slow progress towards speciation of relictual populations in Andean valleys can explain why subspecies are so diverse in the Amazon, while young species are commoner in the more species-rich Andes.

Probably, therefore, some combination of explanations underlie the discrepancies in diversity pattern between species and subspecies. On the one hand, ecotones are probably steeper in the Andes, and the terrain is more conducive to geographic isolation. This contributes to a greater tendency to transition across the species boundary. On the other hand, if the older ages of subspecies characteristics found in Andean *H. erato* (Hines et al. 2011) are general among the heliconiines, many of the "young species" found in the Andes may in fact be relictual "older subspecies" left behind after rapid competitive spread of new subspecies variants from the Amazon. If so, the Amazon may be the "species pump", while the Andes has high diversity because it is a "museum" of subspecies that have transitioned the species boundary more often due to their greater age. This in effect could reverse the directionality of Fjeldså's (1994) argument, by focusing on sources of speciation in processes below the species level.

Conclusions

I found that the heliconiine butterflies in species rich regions tend to have short phylogenetic branch lengths, and species in depauperate regions tend to have long branch lengths. This result supports a role for variation in speciation and/or extinction rates in driving the latitudinal species richness gradient, rather than just evolutionary age or phylogenetic niche conservatism. An apparent mismatch between areas of high species richness and those inferred to be favourable for speciation because of elevated

subspecies richness, polymorphisms, and the presence of suture zones, suggests that speciation is more likely to be completed in Andean foothills than in the Amazon lowlands. In summary, most heliconiine species originated in the upper and middle Amazon basin and the eastern slopes of the Andes in Colombia, Ecuador and Peru, and these are areas that have the highest current species richness for this group.

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Chapter 3. Jordan's Law refuted? The geography of speciation in heliconiine butterflies.

Abstract

Sympatric speciation has been the subject of considerable debate during the last hundred years, with many biologists doubting its plausibility both for empirical and theoretical reasons. Recent mathematical models have supported the theory of sympatric speciation and there is evidence for some cases**,** but how common the process is remains unanswered. Here, I estimate the frequency of sympatric speciation in *Heliconius* butterflies and their allies (Lepidoptera: Nymphalidae: Heliconiina). The observed frequency of sister species showing geographic range overlap is compared with expectations generated via simulations of the geography of speciation. In the simulations I vary the proportion of sympatric events and species' ranges follow a random walk, allowing them to shift, grow and contract. I find that the geographic ranges overlap in 8-9 cases of 21-23 heliconiine sister species pairs (depending on species concept); a much higher proportion than reported in birds and mammals. I show that the patterns of range overlap are most consistent with simulations in which sympatric speciation is common, contributing 35%-90% of speciation events. However, a scenario not accounted for in the simulations, parapatric speciation followed by a tendency for daughter species to expand rapidly into one another's ranges, presents a highly plausible alternative explanation. The high levels of overlap contradict the long held tenet of biogeography that closely related animal species are usually allopatric, but whether heliconiines simply represent the exception that proves the rule will require biogeographic comparative studies for a wider range of animal species than have been considered to date.

Introduction

Despite a controversial history, sympatric speciation is now generally seen as theoretically possible (Dieckmann and Doebeli 1999, Gavrilets and Waxman 2002, Gavrilets 2004) and, at least under a biogeographic definition there are cases where it seems the most likely explanation (Sorenson et al. 2003, Barluenga et al. 2006, Savolainen et al. 2006). Consequently, much of the debate has shifted to the relative importance of different geographic modes of speciation (Jiggins 2006).

The geographical distributions of contemporary sister-species, especially the degree of range overlap, have been used to infer dominant modes of speciation at least as far back as the turn of the twentieth century (Jordan and Kellogg 1907) and variants on this approach continue to be used today (Kisel and Barraclough 2010, Papadopulos et al. 2011). In recent years as molecular sequence data have become available, the relationship between range overlap of sister taxa and time since speciation (the age-range correlation) has been used to infer the dominant geographic mode of speciation in a clade: if most speciation is allopatric then recently diverged sister taxa should tend to be allopatric, with increasing sympatry between older taxa attributable to post-speciation range movements. Alternatively, if most speciation is sympatric, the expectation is that recently diverged species will tend to be sympatric and sympatry between older species-pairs will be reduced (Lynch 1989, Chesser and Zink 1994, Barraclough et al. 1998, Berlocher 1998, Barraclough and Vogler 2000, Fitzpatrick and Turelli 2006, Perret et al. 2007).

Unfortunately, studies based on age-range correlations have often been inconclusive because a mixture of allopatric and sympatric speciation tends to produce an age-range correlation that is indistinguishable from the pattern left by a single mode of

speciation followed by extensive range movement (Barraclough and Vogler 2000, Losos and Glor 2003, Fitzpatrick and Turelli 2006, Perret et al. 2007). Phillimore et al. (2008) used spatially explicit simulations of speciation and subsequent stochastic movement of the daughter species' ranges and found that, when considered together, the proportions of sister species showing zero or complete range overlap and their degree of bimodality are more informative than the age range correlation about the relative frequencies of allopatric versus sympatric speciation. On applying this approach to data on the geographic distributions of sister species of birds, Phillimore et al. (2008) found that the observed patterns of range overlap were consistent with simulations in which allopatric speciation predominates and sympatric speciation contributes no more than 5% of speciation events. Here, I apply the same approach to heliconiine butterflies. I adopt the normal biogeographic definition of sympatric speciation (i.e. sympatric speciation is said to occur when the diverging species have overlapping geographic ranges). In any case, maps of species range provide little information on interdemic gene flow (*m*), used in alternative "population genetic" definitions (Fitzpatrick et al. 2008, Mallet et al. 2009).

Few previous studies have tested the geography of speciation in taxa thought to be likely candidates for sympatric speciation (Berlocher 1998, Barraclough and Vogler 2000, Linnen and Farrell 2010). Heliconiine butterflies fulfil two conditions that should be conducive to sympatric speciation. First, adaptive traits (wing colour patterns involved in Müllerian mimicry) are used also in mate recognition and are frequently correlated genetically with mate preference (Kronforst et al. 2006, Chamberlain et al. 2009, Merrill et al. 2011). These are so-called "magic traits" that have pleiotropic effects on both local ecological adaptation and mate choice (Gavrilets 2004, Servedio et al. 2011). It is therefore conceivable that reproductive isolation in

sympatry could result from a switch in mimicry, circumventing the impediment to divergence that recombination usually poses (Felsenstein 1981, Gavrilets 2004). Second, heliconiines are phytophagous and highly host-specific, the majority feeding on host-plants from the family Passifloraceae. In species that exhibit host plant fidelity and mate on their hosts, reproductive isolation may arise after a host plant shift, hence it is perhaps no coincidence that many of the most compelling cases of sympatric speciation involve phytophagous insects (Bush 1969, Drès and Mallet 2002, Berlocher and Feder 2002). Heliconiines males frequently patrol host plants and monitor larvae and pupae they find there, with mating often taking place on or near the host (Mallet 1986, Estrada and Gilbert 2010). Furthermore, 42% of species in the genus *Heliconius* are known to engage in "pupal mating", where mating sometimes occurs before females have fully emerged from their pupae (Gilbert 1991, Deinert et al. 1994). Thus, shifts in host plant use would likely generate some reduction in gene flow that could be important in speciation.

I assess evidence that wing pattern transitions and host shifts play a role in heliconiine speciation by calculating the proportion of sister species that differ in wing colour pattern or host use. However, gradual post-speciation divergence of either trait may also cause sister species to differ (Jiggins et al. 2006), in which case we would expect younger sister species to be more similar in colour pattern and host use than older sister species. I test for this effect by regressing sister species' colour pattern / host plant differences on phylogenetic branch length; the slope will estimate the temporal trends in colour/host divergence and the intercept will estimate the degree of colour/host divergence expected at speciation (assuming that species traits do not diverge so rapidly as to obscure the relationship between trait and branch length). Sympatric sister species might also be expected to be more different ecologically than

allopatric sister species. This is expected following sympatric speciation (where the divergence can either be seen as generating reproductive isolation itself, or facilitating species coexistence after speciation) and also under secondary contact (when species should be ecologically divergent in order to coexist). I test for this by examining the correlation between geographic range overlap and ecological similarity.

A subsidiary aim of this paper is to investigate the effect of the practical application of different species concepts on inferences of geographic modes of speciation. Each species concept places a different emphasis on geographical taxa. For instance, with molecular tools taxa identified as species under the diagnostic version of Phylogenetic Species Concept (PSC) can be identified readily in allopatry, whereas biological species can be identified conclusively via reproductive isolation in sympatry only. I test the effects of two versions of the biological species concept (BSC), and carry out some analyses using a "diagnostic version of the phylogenetic species concept (PSC) as follows: (1) Under a “strict” biological species concept, species are defined as groups of interbeeding populations that are reproductively isolated from other such groups (Mayr 1995). Here “semi-species” which hybridize relatively frequently at parapatric boundaries, or are inferred to be likely to do so if they occur in complete allopatry are lumped into the same species as their closest relatives; (2) Under a “relaxed” biological species concept, species are characterised by substantial but not necessarily complete reproductive isolation (Coyne and Orr 2004), with disjunct or parapatric semi-species considered full species, as in current heliconiine taxonomy (Brown 1981, Lamas 2004, Rosser et al. 2012). Relaxed biological species correspond approximately to those recognised as separate genotypic clusters (Mallet 1995), for example where hybrids or intermediates between taxa may occur, although are relatively rare in a well-studied zone of parapatric overlap (Jiggins et al. 1997, Jiggins

and Mallet 2000). (3) Under the diagnostic PSC, traditional heliconiine subspecies differing in fixed, diagnostic colour pattern traits would likely be given full species status (Cracraft 1989). Although full phylogenetic information on these taxa are not available, I use geographic distribution data to investigate the likely outcome of adopting a diagnostic species concept by measuring the proportion of each subspecies range that overlaps (i.e. where it is polymorphic) with other subspecies.

The aims of this paper are therefore threefold: 1) To estimate the relative importance of different geographic modes of speciation in heliconiines. 2) To test whether shifts in ecology are associated with speciation, and whether these events are consistent with sympatric speciation. 3) To investigate the influence of different species concepts on estimated relative importance of sympatric and allopatric speciation.

Methods

I compiled a database of 58,059 locality records for 70 species and 431 subspecies of heliconiines, and mapped the species and subspecies distributions using α-convex hulls to convert the point localities into vector polygons projected in a Lambert Cylindrical Equal Area projection (Edelsbrunner et al. 1983). I describe the dataset and mapping procedure in detail in chapter 2. In the present study, I included an additional sister species pair of heliconiines not mapped in chapter 2; *Philaethria dido* and *Philaethria ostara* cf. *diatonica*, which were mapped using locality records published in (Constantino and Salazar 2010). I applied three taxonomies, corresponding to a relaxed biological species concept (Coyne and Orr 2004), a strict biological species concept, and a diagnostic version of the phylogenetic species concept. Relaxed biological species designations followed Lamas (2004) with certain exceptions; table A2.1 in Appendix 2 indicates where our taxonomy differs. Relaxed

sister pairs (table A2.2) are given by Beltrán et al. (2007), with the following exceptions: i) in the absence of phylogenetic information, when a genus contained only two species they were assumed to be sisters (*Agraulis* and *Podotricha*). ii) I treated *Heliconius erato* and *Heliconius chestertonii* as separate sister species (Arias et al. 2008). I split *Heliconius demeter* into *H. demeter* and *Heliconius sp. nov. (c.f. eratosignis*); the two are sister species that overlap in N.E. Peru according to recent molecular data (Dasmahapatra *et al.* in prep.). iii) Finally, I did not use the putative sister grouping of *Heliconius himera* and *Heliconius hermathena* indicated by Beltrán et al. (2007) given phylogenetic uncertainty regarding the origins of *H. hermathena* (Jiggins et al. 2008). Strict biological species (table A.2.3) were defined on the basis of ability to overlap broadly without frequent hybridisation between sister species (Mallet et al. 2007), with sister comparisons made by collapsing phylogenetic nodes of affected allopatric relaxed biological species (table A.2.4). In order to investigate the probable outcome of adopting a phylogenetic species concept, for every polytypic species we calculated the proportional overlap of each subspecies with its conspecifics.

I quantified overlap between sister species as the area of sympatry divided by the area of the smaller species range, giving an index ranging from 0-1 (Anderson and Evensen 1978). To take account of the geographical incompleteness of sampling and small inaccuracies in the mapping procedure, I define < 0.05 overlap as complete allopatry and > 0.95 overlap as complete sympatry. Bimodality of data was quantified as (z x c)/(a x b), where z and c are the number of cases of complete and zero overlap observed, and a and b are the numbers of cases of complete and zero overlap that would occur if all the data were split evenly between completely and non-overlapping sister pairs (Phillimore et al. 2008). The observed data were compared with values

generated from simulations using a two-tailed test; parameters were considered to have been unlikely to give rise to the observed data if observed values fell outside the 2.5 and 97.5 percentiles of the simulated distribution. I then eliminated parameter combinations for which any of the observed values for the three indices was unlikely to arise.

Simulations

The simulation-based approach employed broadly follows Phillimore et al. (2008). Here, I give an outline of the approach. Each replicate set of simulations modelled speciation as many times as there are pairs of sister species. I ran 1000 replicates for each possible combination of parameters and explored all possible proportions of sympatric speciation events. Speciation was simulated by randomly dividing the parental species' geographic range into two daughter ranges, whose positions relative to one another depended on the mode of speciation being employed (allopatric or sympatric). When simulating sympatric speciation, the smaller daughter range was placed randomly within the larger daughter range. For simulations of allopatric speciation, we varied the geographic configurations of the ranges (vicariant, peripatric and parapatric). When simulating peripatric speciation, I defined the size of the smaller range as 5% of the starting range size. Species' ranges were rectangular and the total area available to ranges was a square grid of a 100 x 100 units. Range movements were simulated by adding a random normal deviate with a mean of 0 at each time step to the vectors corresponding to the top, bottom, right and left extents of each species range. Different rates of range change were explored by varying the standard deviation of distribution from which the values were drawn, we used 0.25, 0.5, 0.75, 1, 1.5, and 2. I also examined the effect of giving the species ranges a tendency to grow by increasing the mean to 0.1. I parameterised the duration of

simulations using mitochondrial DNA divergence-based branch lengths of sister species estimated using a relaxed clock method with a multilocus sequence-based phylogeny as a relative estimate of time since speciation (Mallet et al. 2007). Branch lengths ranged from 0.88- 25.86% divergence; I multiplied these values by 10 to give the number of time steps for each simulation. When I lacked information on branch lengths (four cases for relaxed biological species, three cases for strict biological species), I used the proximate ancestral node to set a maximum time of divergence for the sister pair, and then for each replicate assigned the branch length as a random draw from a uniform distribution between 0 and the maximum time for divergence. I used heliconiine species range sizes to set the size of the initial geographic range relative to the total area available for species ranges during simulations. I defined the area available to heliconiines as the total area occupied by the sub-tribe. I used the median range size of all heliconiine species (6.5% of the total area for relaxed biological species, 13.4% for strict biological species) and the median range size of all sister species (16% relaxed BS, 17.6% strict BS) as starting range sizes in simulations. I also ran simulations with starting ranges double these sizes (because the simulations set the starting sizes of the daughter ranges by dividing the initial range size in two).

Age-range correlations

I also tested for an age-range correlation, using ordinary least squares regression with geographic overlap as the dependent variable, and molecular phylogenetic branch length as the predictor. Intercepts were fitted by linear regression of the arcsine-transformed proportion of sympatry (Barraclough and Vogler 2000). Unlike most previous analysis, I included only sister species in the regression, thus avoiding the problem of reconstructing ancestral ranges for comparisons within the phylogeny.

Sister species without branch lengths estimates were excluded from the age range correlation.

Ecological divergence

I classified heliconiine species wing colour patterns (Table A.2.5 in appendix 2) using an updated and modified version of the colour pattern scheme presented by (Turner 1976, Brown 1981)**.** This scheme classifies colour patterns into broad groups (e.g. black with yellow forewing band and red hind-wing band). These broad colour pattern classes may then be subdivided into further mimicry rings, but speciation seems more likely to be driven by major shifts in colour pattern rather than minor variations (Jiggins et al. 2001). I used Beccaloni et al. (2008) as the principal source for host plant records. I excluded all records marked as dubious, and all those known or thought to have been recorded from captive populations. I also excluded all records where the host plant identification was marked as doubtful. If a host plant species was identified as "near" to a known species, it was treated as a separate species. I measured the similarity of colour patterns and host plants between pairs of species using the Jaccard similarity coefficient; if species *a* and *b* share *x* colour patterns, and comprise a total of *y* patterns, then their colour pattern similarity is x/y.

I then applied three approaches to relaxed biological species to test whether shifts in ecology might be important for speciation. Firstly, I simply counted the number of sister species showing no overlap in colour patterns and host plants. Secondly, for the sister pairs with available molecular phylogenetic information, I used a general linear model with binomial errors to investigate the relationship between the Jaccard similarity coefficient and phylogenetic branch length. Due to overdispersion I corrected the standard errors using a quasi-GLM model where the variance is given by

ϕ x μ, where μ is the mean and ϕ is the dispersion parameter. If divergence in colour and host plant were gradual and independent of speciation, we would expect to estimate an intercept of 1 and a significant negative correlation (i.e. the most recently diverged sister species share the same colour patterns / host plants, with older sister species exhibiting decreasing similarity in these traits). Alternatively, under a punctuated model where speciation is accompanied by divergence, we would expect to estimate an intercept closer to zero (i.e. the most recently diverged sister species already have different colour patterns / host plants). To test whether the degree of ecological similarity is related to the degree of overlap, overlap was included as an additional term in the model. I also examined the correlation between ecological similarity and overlap using all possible pairwise combinations of species in the phylogeny. I also tested whether these ecological traits are dispersed or clustered across the phylogeny via a Mantel test applied to pairwise comparisons of ecological similarity and phylogenetic distance for all species in the phylogeny. A negative correlation indicates that closely related species tend to have similar ecological traits, conversely a positive correlation indicates that closely related species tend to be more divergent in ecological traits than distant relatives are.

Results

Range overlap of heliconiines

The distribution of each smaller-ranged heliconiine species overlaps (overlap > 0.95) with its sister in 8 out of 23 cases under a relaxed BSC and 9 out of 21 cases under a strict BSC, respectively (Figures 3.1,3.2 A.2.1). The number of non-overlapping pairs (overlap < 0.05) is more strongly influenced by species concept, with six sister pairs non-overlapping under a relaxed BSC and only three pairs non-overlapping under a

strict BSC. Accordingly, the bimodality score for overlap (a high score would have sister pairs divided evenly with half overlapping and half non-overlapping) was higher for relaxed biological species (0.36) than for strict biological species (0.25). In contrast to the patterns observed for biological species, there are more allopatric phylogenetic species (i.e. subspecies) (129 cases) than sympatric phylogenetic species (79 cases).

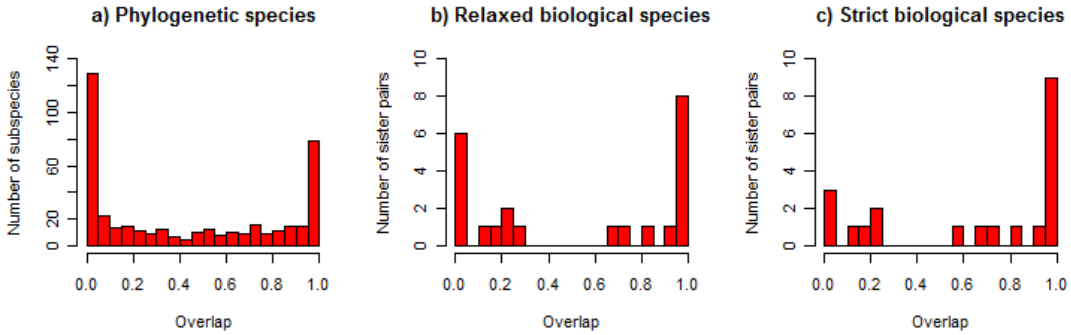

Figure 3.1. Range overlap in heliconiine butterflies. Histograms of range overlap for a) phylogenetic species (=subspecies), b) relaxed biological sister species, and c) strict biological sister species.

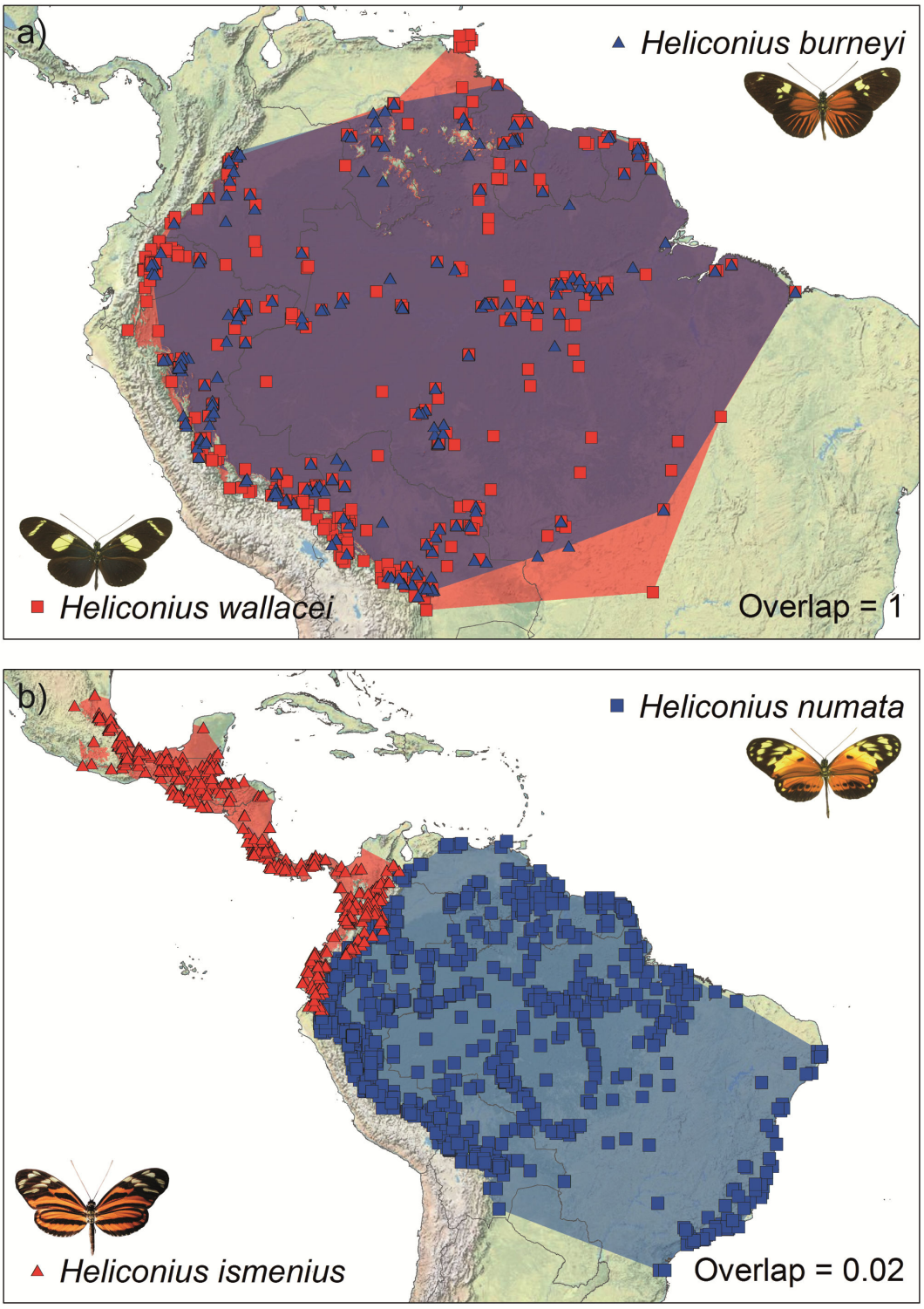

Figure 3.2. Examples of sympatric sister species (a) and allopatric sister species (b).

Simulations

To quantify the relative proportions of different geographic speciation models underlying the observed patterns of range overlap in heliconiines I compared these empirical results to those generated using simulations of geographic speciation and stochastic post-speciation range dynamics. In the simulations I varied the proportion of speciation events that involved sympatric versus non-sympatric geographic ranges (vicariant, peripatric or parapatric), the rate of stochastic post-speciation range movement and the tendency for ranges to grow. Results show that when most speciation was non-sympatric, sister species with completely overlapping ranges were always rare (Figs. 3.3A & 3.4A). This is because even extensive range movements will rarely bring allopatric or parapatric sister species into complete sympatry. In contrast, the number of cases of non-overlapping sister species varied substantially, because even small range movements can easily lead to sister species with some geographic overlap (Figs. 3.3B & 3.4B). In simulations where most speciation was sympatric, non-overlapping sister species were rare, because only extensive range movements are likely to make species that arose in sympatry non-overlapping (Figs. 3.3B & 3.4B). However, the number of cases of sympatric sister species was very variable, because even small range movements will often move species ranges out of complete range overlap (Figs. 3.3A & 3.4A).

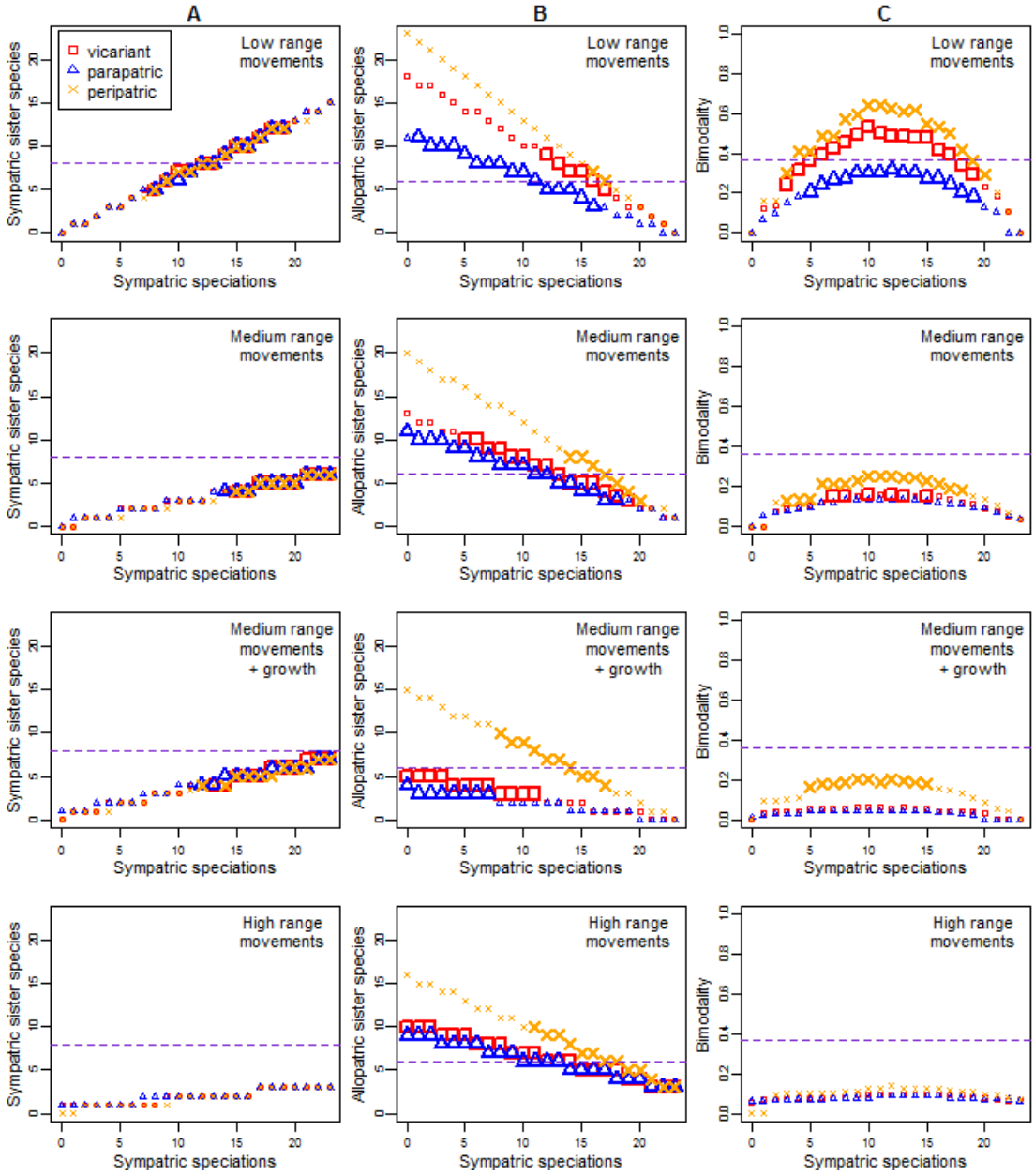

Figure 3.3. Results of simulations of relaxed biological species for selected parameters. The x-axis shows the number of sympatric speciation events, the y-axis shows the median number of completely overlapping species (column A), the median number of non-overlapping species (column B) and the median bimodality (column C). The dotted purple line shows the values observed for heliconiines. Simulations were run using a medium sized range (1595), while varying the rate of range movements and the tendency of ranges to grow following speciation. The geographic configuration of the allopatric range (vicariant, parapatric or peripatric) is shown in the key. Simulations results that were not significantly different from the observed values are indicated with bold symbols.

Simulations showed that the observed numbers of overlapping sister species pairs were unlikely ($P < 0.05$ under a two-tailed test) to arise when sympatric speciation comprises less than 35% (relaxed BSC) or 43% (strict BSC) of all speciation events. In contrast, the observed numbers of non-overlapping pairs frequently arose in simulations with all proportions of sympatric speciation (although not under all combinations of parameters). The bimodality scores of the observed data were unlikely to arise in simulations where either non-sympatric or sympatric speciation predominated (Figs. 3.3C, 3.4C), and are consistent with simulations where 13-87% of speciation was sympatric under a relaxed BSC, and 0-90% under a strict BSC. Overall, the simulations that produced combined results not significantly different to any of the three observed indices had frequencies of sympatric speciation between 35%-78% (relaxed BSC) or 43%-90% (strict BSC), low to moderate range movements (0.25-1.5, relaxed BSC; 0.25-1, strict BSC), either zero or positive range growth, and where the non-sympatric speciation events were any of vicariant, parapatric or peripatric (Tables A2.6, A2.7 in Appendix 2). However, when the non-sympatric speciation was vicariant or parapatric, the observed patterns were obtained only when if the range growth parameter was set to zero. This is because a tendency for ranges to grow frequently results in overlap between vicariant / parapatrically derived sister species. All starting range sizes were able to generate the observed data, but when starting range sizes covered a larger proportion of the simulation domain area, the observed data were likely to arise (i.e. $P > 0.05$) under a wider range of conditions.

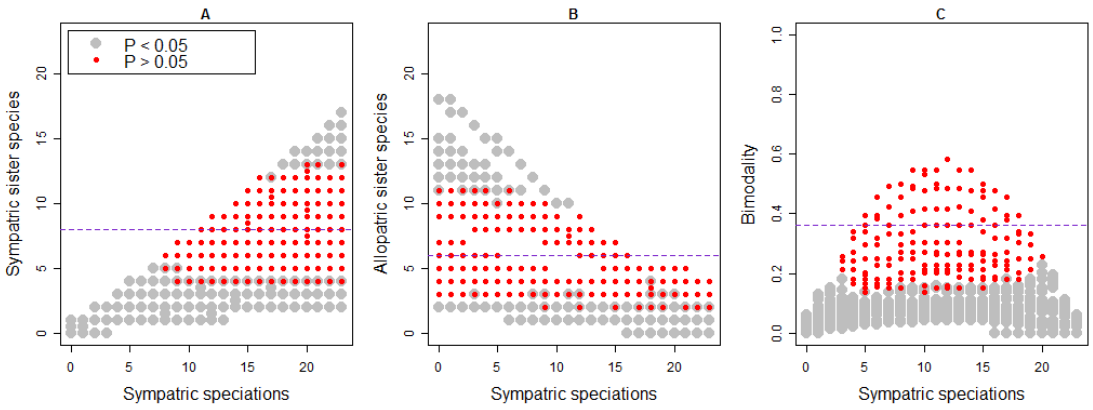

Figure 3.4. Results of simulations for 23 pairs of relaxed biological species for all combinations of parameters. The x-axis shows the number of sympatric speciation events, the y-axis shows the median number of completely overlapping species, the median number of non-overlapping species and the median bimodality. The dotted purple line shows the values observed for heliconiines. The smaller red dots show simulation results that were not significantly different from the observed values, the larger grey dots show simulation results that differed significantly from the observed values.

Age-range correlations

To facilitate comparisons with previous work, I conducted age-range correlations for relaxed and strict biological species. Scatter plots of the correlations are shown in figure 3.5. The intercepts of the models had intermediate values (values on arcsine scale - relaxed biological species: intercept = 0.48 ± 0.23 (0.46 backtransformed), slope = 0.05 ± 0.03 (0.05 backtransformed); strict biological species: intercept = 0.8 ± 0.23 (0.72 backtransformed), slope = 0.03 ± 0.03 (0.03 backtransformed). To test whether species ranges show a tendency to expand after speciation, we examined the correlation between the range size of relaxed biological sister species and phylogenetic branch length. The size of the smaller sister species' range size was positively related to branch length (OLS: log(range size) = 11.35 + 0.27 branch length, $p < 0.05$, $r^2 = 0.30$), but the larger range size was not (log(range size) = 14.65+ 0.11 branch length, ns, $r^2 = 0.11$).

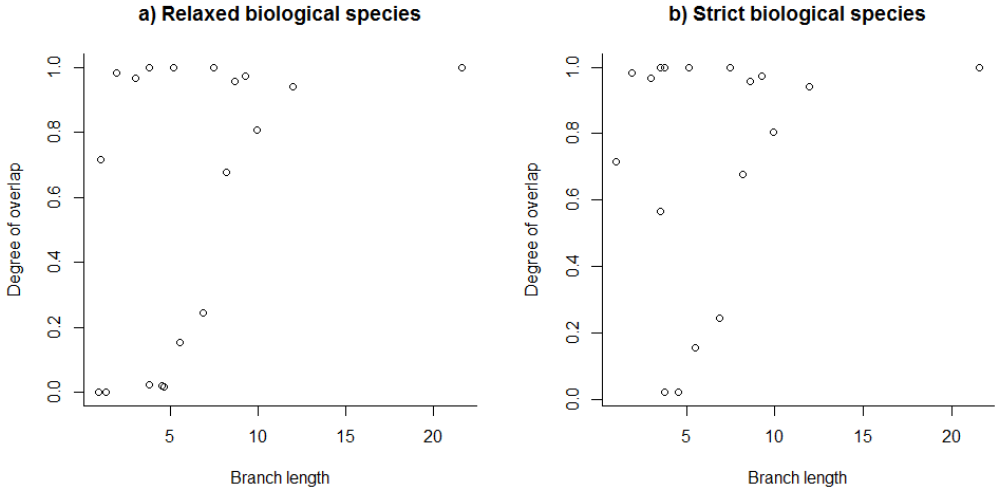

Figure 3.5. Age-range correlation analyses for a) relaxed biological species and b) strict biological species.

Ecological divergence

To test whether heliconiines species exhibit divergent ecology which would support the plausibility of sympatric speciation, I examined whether closely related species tend to share colour patterns and host plants by calculating the Jaccard's similarity coefficient for pairs of relaxed biological species (low scores indicate that pairs of species share few colour patterns / host plants; high scores indicate that they share many). 13 of 23 sister species pairs have no colour patterns in common (Fig. 3.6, Table A.2.5). For the relationship between sister species' colour pattern similarity and phylogenetic branch length (figure 3.7A), I estimated a positive but non-significant slope (= 0.20 ± 0.12, values on logit scale) and an intercept close to zero (= -2.65 ± 0.94 on the logit scale or 0.07 as a proportion). I identified no effect of sympatry on colour pattern similarity, when the contemporary geographic context of sister pairs was included as an additional predictive term in the model. Across all pairwise comparisons among heliconiine species in the phylogeny, colour pattern similarity is negatively correlated with phylogenetic distance (Mantel test; 10,000 permutations, *r*

= -0.10, P < 0.001; figure 3.8A). I found no correlation between range overlap and colour pattern similarity (Mantel test; 10,000 permutations, r = 0.14, P = 1).

None of the heliconiine sister species with available data have been recorded feeding on exactly the same set of host plant species; 6 of 17 sister species with available data use entirely different hosts, and the remaining sister pairs have low Jaccard similarity scores (<0.5) (Fig. 3.6). For the relationship between sister species' host plant similarity and phylogenetic branch length (figure 3.7B), I estimated a positive and significant slope (=0.12 ± 0.04, values on logit scale, p < 0.01) and an intercept close to zero (=-3.32 ± 0.50 on the logit scale or 0.03 as a proportion, p<0.001). The degree of sympatry was not correlated with host plant similarity when included as an additional term in the model. Across all species in the phylogeny there was no significant correlation between host plant similarity and phylogenetic distance (Mantel test; 10,000 permutations, r = -0.08, p = 0.90; figure 3.8), or host plant similarity and range overlap (Mantel test; 10,000 permutations, r = 0.01, P = 0.62).

Figure 3.6. Heliconiine phylogeny based on nuclear and mitochondrial DNA loci (Beltrán et al. 2007), but with mitochondrial DNA divergence-based branch lengths, adapted from (Mallet et al. 2007), with sister pairs of relaxed biological species in black. Photos show example phenotypes for sister pairs; the upper species in each pair is shown in the photo to the left and the lower species in the photo to the right. The proportion of the smaller range that overlaps the larger range is shown in black in the pie chart. Host plant use shows the number of host plant species used by the upper species in black and the number used by the lower species in grey, with the number of host plant species shared by the sisters in stripes. Nodes in the phylogeny marked with an asterisk indicate sister species pairs for which branch length data were not available for one of the sisters; in these cases the split was placed midpoint along the branch leading to the sister with known branch length. These species pairs were not included in regressions of ecological traits against branch lengths.

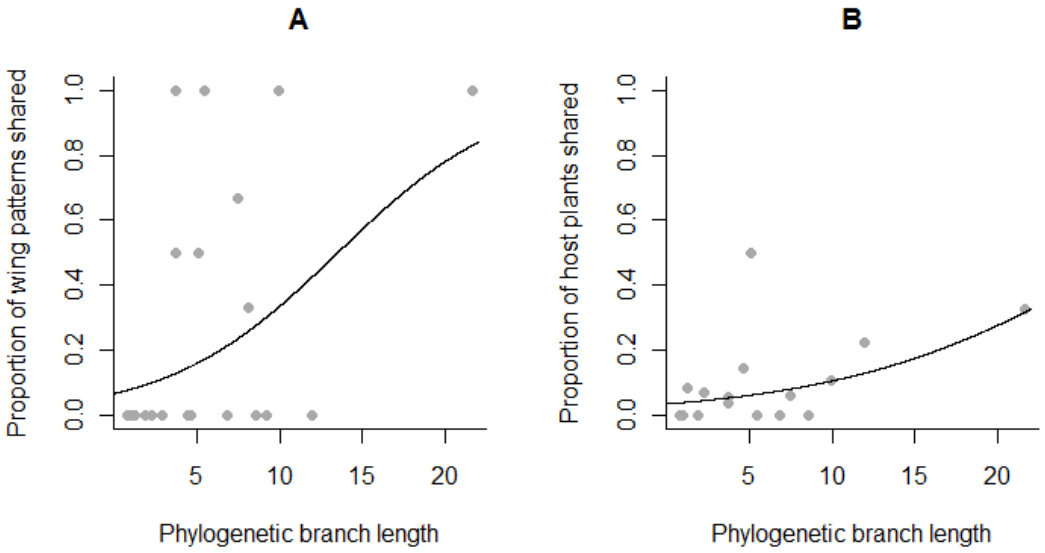

Figure 3.7 –Sister species' wing colour pattern (A) and host plant (B) similarity plotted against phylogenetic branch length.

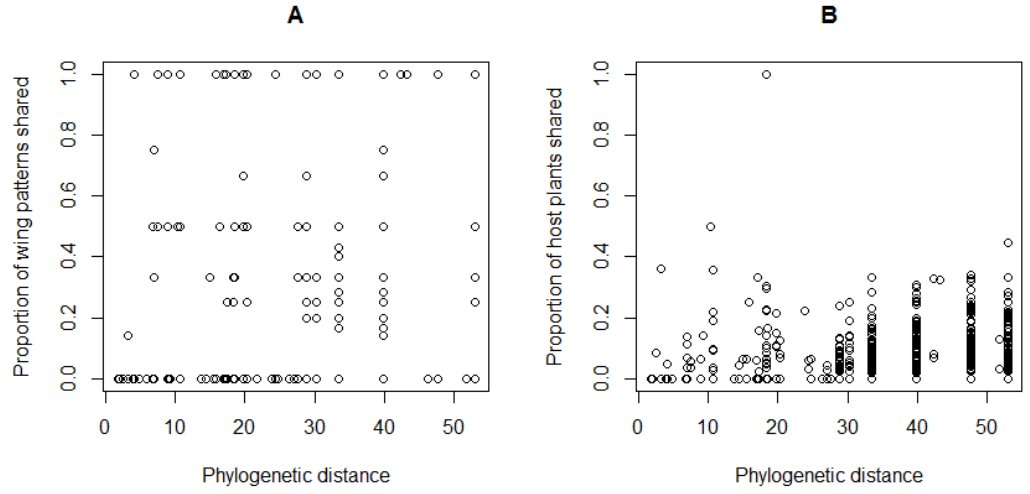

Figure 3.8. Pairwise comparisons of wing colour pattern similarity (A) and host plants (B) across all species in the heliconiine phylogeny. A weak negative trend (r=-0.10) was apparent in A, no trend was apparent in B.

Discussion

I found that up to 43% of heliconiine sister species to be entirely sympatric, and up to 66% have overlap > 0.5, depending on the species concept employed. This finding is in contrast to birds (5% show complete overlap as defined here (Phillimore et al. 2008)) and mammals, where sympatric sister species are also rare (14-23% show overlap > 0.5 (Fitzpatrick and Turelli 2006)). The age-range correlations for heliconiines had intercepts at intermediate values, which could be either interpreted as the product of range movements post speciation, or a mixture of allopatric and sympatric speciation (Barraclough and Vogler 2000, Losos and Glor 2003). However, the simulations revealed that the high proportion of sympatric sister species observed in heliconiines is unlikely to arise without sympatric speciation events, even if range movements have been extensive. In fact, of the scenarios covered in my simulations, the observed overlap among heliconiine sister species is most consistent with a model in which sympatric speciation is common, contributing between 35% and 90% of all speciation events. Although the inference of a high frequency of sympatric speciation seems exceptional, genetic linkage between wing colour patterns and mate preference in heliconiines may mean that it is not implausible (Kronforst et al. 2006, Chamberlain et al. 2009, Merrill et al. 2011). In addition, recent genomic studies suggest that a number of *Heliconius* species (e.g. *Heliconius elevatus* and *Heliconius heurippa*) may have arisen following adaptive introgression of colour pattern elements (Mavárez et al. 2006, Jiggins et al. 2008, Dasmahapatra et al. 2012); such hybrid speciation necessarily requires sympatry between the parental species, although the simulations do not specifically model hybrid speciation. A possible case of incipient sympatric speciation has also been documented; in western Ecuador white

and yellow colour morphs of *Heliconius cydno alithea* exhibit weak assortative mating (Chamberlain et al. 2009).

A critical assumption of the simulations employed here is that they assume species ranges to move independently and stochastically following speciation. In reality, this assumption is unlikely to hold. For instance, closely related species will often be ecologically similar, so that competition may limit secondary sympatry (Hardin 1960). Alternatively, if speciation involves a shift in host plant or other ecological dimension, competition between sister-species would become relaxed and there could be a rapid sympatric range expansion of the new species. As climatic niches tend to be conserved in sister species (Peterson et al. 1999), this could lead to acquisition of almost identical ranges by recently diverged sister allospecies. In heliconiines the initial stages of speciation may therefore involve parapatric or allopatric divergence of a population to specialise on an alternate host plant, followed by rapid range expansion and geographic overlap with the sister (or parent) taxa. A strong positive correlation between the range size of the smaller ranged species and phylogenetic branch length was also noted, consistent with the hypothesis that rapid range expansion follows speciation (although this finding does not discriminate sympatric and parapatric origins, as rapid range expansion is also expected following sympatric speciation). Parapatric speciation may represent a more likely hypothesis than pure allopatric speciation, because vicariant and peripatric models of allopatric speciation specify geographic barriers preventing gene flow. Such barriers are rare for heliconiines; in tropical America the Andes form the only major barrier (Chapter 2). Case studies of heliconiines known to have high hybridisation rates, but which are considered separate 'relaxed' biological species because of bimodal distributions of

genotypes also often involve parapatric distributions (Jiggins et al. 1997, Jiggins and Davies 1998, Gilbert 2003, Arias et al. 2008).

The lack of a robust densely-sampled subspecies-level phylogeny precluded simulations of the geography of speciation using a diagnostic phylogenetic species concept based on fixed colour pattern differences (Cracraft 1989). However, most heliconiine subspecies do not overlap with their consubspecifics, and so the conclusions drawn about the relative frequencies of different modes of speciation from species ranges are likely to be highly sensitive to the species concept applied. The relative paucity of sympatric subspecies also runs counter to expectations if speciation frequently occurs sympatrically via colour pattern shifts, as under this model colour pattern polymorphisms should be fairly common. Subspecies are usually defined as geographic variants that are relatively constant over large areas (Mallet 2001), which will lead to some bias against classifying local morphs in polymorphic populations as phylogenetic “species” under a diagnostic criterion. Nonetheless, in some highly polymorphic heliconiines "weak subspecies" have been recognized, in spite of frequent local polymorphisms, for example in *Heliconius numata* (Brown 1976). In the case of *H. numata*, such polymorphisms are now known to be due multiple sites within small inversions that trap colour pattern variation in supergene allelomorphs (Joron et al. 2011).

I found no significant correlation between colour pattern similarity and phylogenetic branch length. Nonetheless, the low intercept and positive slope estimated is still reasonably consistent with the interpretation that changes in wing colour pattern are associated with speciation and therefore that sympatric speciation could have occurred following a mimicry shift (Jiggins et al. 2001). A weak negative trend was observed

between colour pattern similarity and phylogenetic distance across all heliconiine species, thus there is some evidence that colour patterns are conserved across the phylogeny. This is probably due to certain colour patterns being restricted to particular clades in the phylogeny. Figure 3.8A shows that despite this overall trend, pairwise comparisons of recently diverged species pairs usually exhibit different colour patterns.

Finding that host plant shifts are associated with speciation events would present an alternative means by which sympatric speciation could occur (Bush 1969, Drès and Mallet 2002, Berlocher and Feder 2002). I found a significant positive correlation between host plant similarity and phylogenetic branch length (on average species that have just diverged were predicted to share just 3.5% of their host plants), which is consistent with host plant shifts being associated with speciation events rather than the simply being the product of gradual divergence. Although I found no correlation between host plant use and phylogenetic distance across the heliconiine phylogeny, this is unsurprising as heliconiine species almost never overlap in host plants (figure 3.8B). Thus, the data are consistent with the hypothesis that host plant shifts occur at speciation.

A further prediction made was that sympatric sister species should tend to differ more ecologically than allopatric species. Ecological differentiation is expected both under sympatric speciation and under secondary contact, as both require ecological differences in order to allow coexistence (Hardin 1960, Coyne and Orr 2004). Shifts in host plants should directly reduce competition between species and allow coexistence. Mimicry shifts could also reduce competition between species and allow coexistence, as there is evidence that heliconiine mimicry rings are segregated to

some extent by habitat (Smiley 1978). I did not find any association between range overlap and host plant use. However, this again is unsurprising; host plant differentiation seems a ubiquitous feature of species differences in heliconiines, irrespective of overlap. Sister species do sometimes share wing colour patterns, yet there was no association between wing pattern similarity and range overlap. Overall, while there is little positive support for the hypothesis of sympatric speciation via mimicry shifts, there are few data that argue convincingly against it.

Conclusions

Naturalists have frequently observed that closely related animal species have allopatric distributions, a pattern believed so ubiquitous in vertebrates that it became known as "Jordan's Law" and was used as evidence against sympatric speciation (Jordan 1905, Jordan and Kellogg 1907). Here, I show that closely related biological species of group of phytophagous insects are often sympatric. In order to place this result in a broader context and establish whether it implies that Jordan's Law can be rejected or is instead a rare exception that proves the rule will require biogeographic comparative studies to be conducted for a broader range of taxa than have been considered to date. The simulations suggest that the range overlaps observed in heliconiines are unlikely to arise as a result of purely allopatric speciation. Unfortunately, as is often the case with studies of the geography of speciation, the observed patterns are consistent with several alternative hypotheses. Finding that heliconiines very rarely use the same host plants suggests that host plant shifts may well be involved in speciation, and presumably facilitate range overlap between species. Mimicry shifts may also be important in speciation, but I did not find any evidence that they are associated with sympatry as is expected by sympatric speciation via mimicry shift.

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Chapter 4. A butterfly hybrid zone correlates with rainfall, contrary to the Pleistocene Refugium theory.

Abstract

I compared the position and shape of two hybrid zones between races of Andean and Amazonian *Heliconius* butterflies in northern Peru from 1986 to 2011. Theory and previous empirical work has suggested that the hybrid zones might be moving towards the Andes. Extensive deforestation and climate change might also be expected to affect their position and shape. However, I found that the hybrid zones have changed remarkably little over the time period. Neither showed evidence of symmetrical widening over the time period, suggesting that they are maintained by selection and are not the product of neutral mixing following secondary contact. I investigated the climatic conditions associated with their position, and find them to be positively correlated with rainfall. I suggest that the exceptionally high levels of precipitation at the edge of the Andes act as a population density trough for butterflies, trapping the hybrid zone at the foot of the mountains. These results diametrically oppose the long-standing Pleistocene Refugium theory, which postulates that the range of each subspecies should be centred on areas of maximum rainfall and with hybrid zones falling in between them.

Introduction

Hybrid zones are narrow zones of phenotypic or genetic change between the ranges of parapatric, genetically distinct forms (Mallet 1986). To determine the fate of hybrid zones it is necessary to study their spatial and temporal dynamics. If hybrid zones represent secondary contact between populations fixed for different alleles that have

equal fitness irrespective of genotype, then the populations may be expected eventually to fuse and lead to a single, possibly polymorphic, species (Endler 1977). If hybrid genotypes have reduced fitness, selection may act to strengthen reproductive barriers across the hybrid zone and continually narrow it until speciation is complete; a process known as reinforcement (Howard 1993). Alternatively, selection can favour interbreeding, leading to the widening and eventual collapse of a hybrid zone (Searle 1993). A third possibility is that a hybrid zone represents an equilibrium situation. Stable hybrid zones can be maintained by exogenous selection (i.e. the fitness of genotypes is dependent on the environment and varies spatially) (Endler 1977). Hybrid zones that are maintained by a balance between dispersal and endogenous selection against introgression (a "tension zone" (Key 1968, Barton and Hewitt 1985)) are also expected to be stable in regions of low population density or dispersal (see below) (Barton and Hewitt 1981, Goldberg and Lande 2007). Finally, a hybrid zone may move, with one form expanding its range at the expense of the other. Finding that a hybrid zones to be mobile is consistent with phase III of Wright's shifting balance hypotheses, where adaptive or more stable genetic equilibria are exported to new populations (Barton and Hewitt 1989).

A number of scenarios predict hybrid zone movement. 1) If one form has a global selective advantage over the other, it will spread through a species range (Fisher 1937). As it is unlikely that two forms have equal fitness, this "advancing wave" scenario seems likely to be common (Hewitt 1988). 2) When a hybrid zone is maintained by exogenous selection, it is expected to track any changes in the environment, such as climate change or habitat alteration. 3) Tension zones are not tied to features in the environment such as ecotones, and any asymmetry in selection between the pure types will cause the tension zone to move (Bazykin 1969, Barton

1979). In addition, asymmetry in dispersal or density between pure types will cause the tension zone to move via "reproductive swamping", in other words, the centre of the zone will receive more immigrants of the more abundant or mobile homozygote and its frequency will rise, pushing the hybrid zone along by weight of numbers. The hybrid zone will therefore move towards areas of low density or dispersal, where it will slow or come to rest due to a reduction in the imbalance between the forms. Such density troughs can be powerful traps and can even prevent the spread of a race with a significant fitness advantage (Barton 1979). 4) Tension zones may also be maintained by frequency dependent selection if the rare form is at a disadvantage (Mallet 1986, Mallet and Barton 1989). With dominance, these hybrid zones can move in the absence of any selective advantage for either homozygote because the mismatch between phenotypic and genotypic clines causes asymmetrical selection. This is because in the centre of the cline allele frequencies are equal, but the recessive phenotype is rarer and selected against. Consequently, the dominant allele expands at the expense of the recessive allele; a process termed "dominance drive" (Mallet 1986, Blum 2002).

I studied the spatial and temporal dynamics of hybridising races of *Heliconius* butterflies in northern Peru. *Heliconius* butterflies are aposematic neotropical butterflies, known for participating in Müllerian mimicry rings (where species share colour patterns in order to share the cost of warning predators as to their bad taste) (Brown 1981). *Heliconius erato* and *H. melpomene* are widely distributed co-mimics that display parallel geographic variation in colour pattern throughout their range, with almost 30 subspecies each currently recognised (Brown 1979, Mallet 1993, Lamas 2004). Races are typically monomorphic within their range but form hybrid zones where they meet. In the upper Huallaga Valley in northern Peru, *H. erato*

favorinus and its mimic *H. melpomene amaryllis* exhibit red patches in the forewings and a yellow hind-wing bar; a pattern known as the "postman" phenotype (figure 4.1). In the adjacent Amazonian lowlands, *H. erato emma* and its mimic *H. melpomene aglaope* are characterised by yellow bands on the forewings and red rays on the hind-wings (the "dennis-rayed" phenotype). Where they meet, a narrow hybrid zone maintained by frequency dependent selection separates the Amazonian and Andean forms (Mallet et al. 1990). Racial variants of the dennis-rayed pattern are found throughout the Amazon basin. However, races displaying variations on the postman pattern occur in disjunct regions around the periphery of the Amazon, from Central America to south-eastern Brazil. Mallet (1993) suggested that this curious distribution might be due to a shifting balance-type process, with the rayed pattern originating in the Amazon and spreading outwards at the expense of the ancestral postman pattern. Recently, molecular evidence has supported this hypothesis; dennis rayed and postman phenotypes have been shown to share a common origin within both *H. erato* and *H. melpomene* (Hines et al. 2011). It remains unclear why the dennis-rayed phenotype might be selectively favoured. One possibility is that the dennis-rayed phenotype is more effective at teaching predators that the bearer is unpalatable. A second possibility is that rayed individuals are selectively favoured because they are protected by a large pre-existing mimicry ring; a number of other species have dennis-rayed patterns (e.g. *H. xanthocles, Neruda aoede* among others). In comparison, the postman pattern is restricted to *H. erato* and *H. melpomene*. Thus dennis-rayed races may benefit from the greater protection conferred by a higher local frequency of dennis-rayed phenotypes. A third hypothesis is that rayed mimicry patterns are superior in lowland Amazonian conditions. For example, a reduction in black scaling compared to Andean races could be involved in temperature regulation (Mallet 1993).

However, because most of the loci determining the colour pattern differences between *H. erato* and *H. melpomene* exhibit near complete dominance, a fourth hypothesis is dominance drive (in which a dominant phenotype has a frequency-dependent advantage in a zone of polymorphism). This alone might explain the spread of the dennis-rayed phenotype. Indeed, based on simulations incorporating dominance drive, Mallet et al. (1990) predicted that the hybrid zone between *H. erato* races in northern Peru should move eastwards at a rate of about 50km per century.

Thus both dominance drive and a selective advantage for the rayed phenotype suggest that the hybrid zone separating rayed and postman races in northern Peru should be moving towards the Andes. In addition, the eastern Andes and adjacent Amazon basin have undergone considerable recent habitat alteration due to deforestation and possibly climate change. Habitat alteration might affect the hybrid zone in a number of ways. If different phenotypes are favoured in different habitats (e.g. closed forest vs. more open areas) (Blum 2008), habitat alteration might alter selective values affecting the two races, enabling one race to increase at the other's expense. Alternatively, habitat loss might reduce predator populations and consequently selection, allowing a widening of the hybrid zone (Mallet and Barton 1989). Finally, increasing temperatures as a response to climate change might effectively "lower" the mountain passes (currently c. 1000m) separating the upper Huallaga valley from the lowlands and allowing increased migration between the two.

In light of this, I compared the position and width of colour pattern clines between 1986 and 2011 using a maximum likelihood based approach. Because habitat loss in the Amazon and climate change in the Andes are not expected to affect the hybrid zone symmetrically, I also test explicitly for asymmetrical changes in cline shape on

either side of the hybrid zones. To identify climatic factors that may be responsible for the hybrid zones position I use generalised linear models.

Methods

Mallet first characterised the hybrid zone between Tarapoto and Yurimaguas in northern Peru in 1984-1987, collecting a total of 1531 *H. erato* from 46 sites, and 874 *H. melpomene* from 45 sites. The majority of specimens were collected in 1986. I re-sampled the hybrid zone between April and October in 2011, collecting 438 *H. erato* and 466 *H. melpomene*. Butterflies were caught using a hand net. A GPS was used to label every specimen with the precise coordinates where it was caught. Mallet's collecting sites were assigned positions along a transect running from 7.324°S, 76.812°W to 5.608°S, 75.903°W (figure 4.1). The transect was chosen to cross the hybrid zone at approximately right angles and to be near to the majority of sites. The positions of collecting sites along the transect were determined by dropping a perpendicular line from each site onto the transect and calculating the distance from the intersection to the transect's start. I followed Mallet's approach but determined the position of individual specimens along the transect, before assigning the specimens into 1km interval "bins" along the transect. Specimens were preserved in NaCl saturated DMSO with Xm EDTA, with the wings removed and kept for reference.

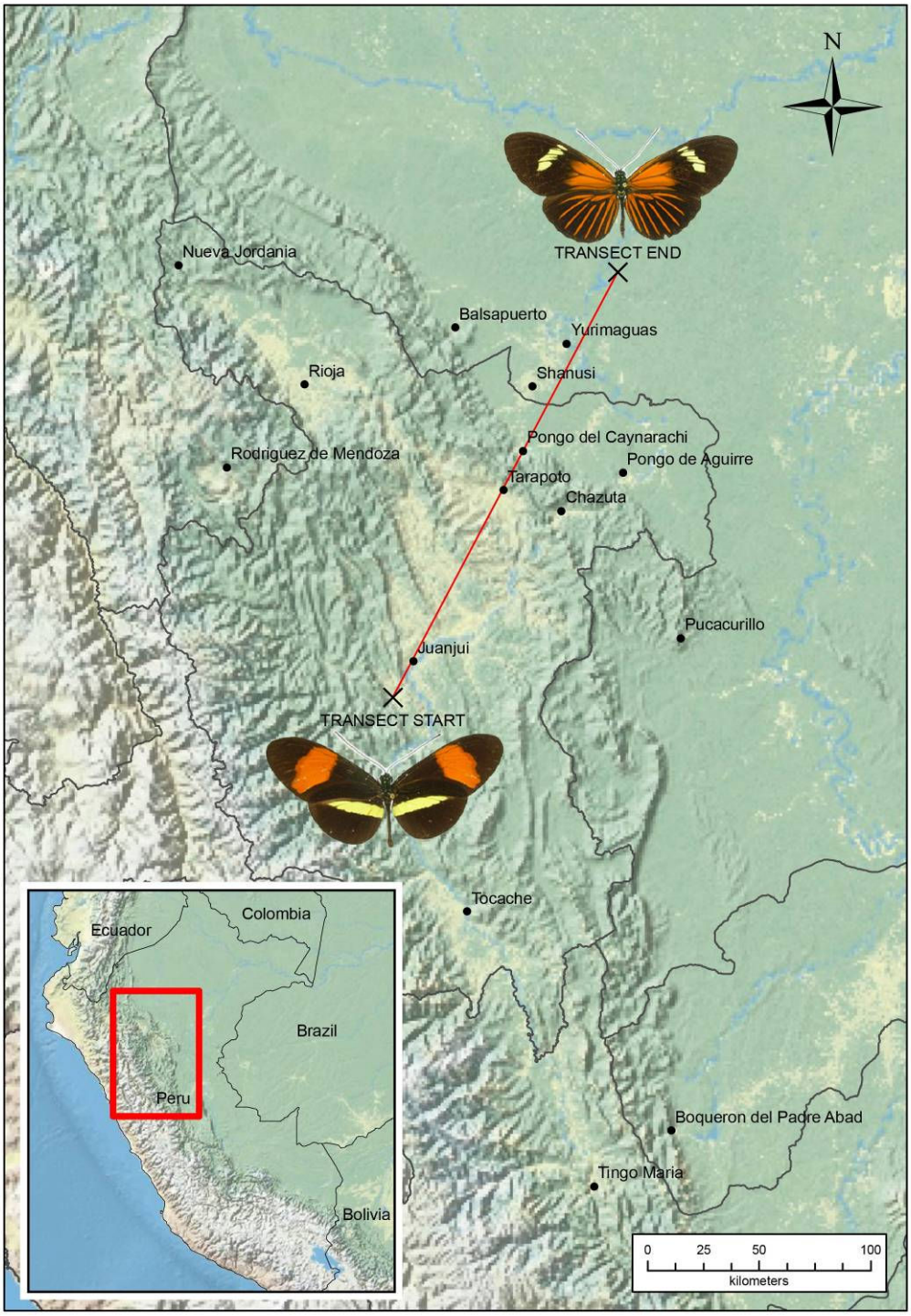


Figure 4.1. Map of the study area. The transect used in Mallet et al.'s (1990) study is marked in red. Examples of the Andean postman phenotype (bottom) and the Amazonian dennis-rayed phenotype (top) are shown.

Specimens were genotyped using their colour pattern phenotypes. The Mendelian genetics of colour patterns in the Amazonian and Andean races of *H. erato* and *H. melpomene* are described in detail in Mallet (1989), and I simply outline them here. In *H. erato*, three loci determine the major colour pattern elements. The *D* locus (cf. D^{Ry}) determines the presence/absence of dennis and rays, and the colour of the forewing band. *DD* individuals have a dennis, rays and a yellow forewing band, whereas *dd* individuals have no dennis or rays and a red forewing patch. Heterozygotes are recognisable because the red colour elements are expressed; *Dd* individuals have a dennis and rays, with a red forewing patch. A dominant locus *S* (cf. *Sd*) determines the width of the forewing band. Recessive homozygotes (*ss*) have a broad forewing band, and dominant homozygotes (*SS*) and heterozygotes (*Ss*) have a narrow forewing band. The *S* locus and the locus *C* (cf. *Cr*) interact to produce the yellow hindwing bar present in postmen. In *sscc* individuals the hindwing bar is completely expressed. In *S-cc* individuals a weak hindwing bar is expressed (with a narrow forewing band), whereas in *ssC-* individuals only the tips of the hindwing bar are expressed (with a wide forewing band). *S-C-* individuals have no hindwing bar expression. In *H. melpomene*, four dominant loci determine colour pattern differences. The *D* locus (cf. D^{R}) determines the presence (*D-*) or absence (*dd*) of the dennis and rays. The *Y* locus (cf. *Yb*) determines the presence (*yy*) or absence (*Y-*) of the yellow hind-wing bar. The loci *N* and *B* interact to determine the shape and colour of the forewing band; the *N* allele codes for a narrow, yellow forewing band, as well as narrowing any red forewing band present. The *B* allele codes for a red band. *bbN-* individuals have a, yellow forewing bar, whereas *B-nn* individuals have a wide, red bar. In *B-N-* individuals a narrow, mixed red/yellow forewing bar is expressed (the narrowing effect of *N* works only on the red band). Finally, double recessive homozygotes

(*bbnn*) have melanic forewings with no band. The *N* and *Y* loci are tightly linked, and the *B* and *D* loci are thought to be moderately or tightly linked (Sheppard et al. 1985, Mallet 1989, Baxter et al. 2010).

Spatial and temporal change

I used the program Cfit-7 (Gay et al. 2008) to fit clines to genotypic (the codominant D locus in *H. erato*) and allelic (the dominant loci) data. For allelic data, I used the Hardy-Weinberg principle to estimate the frequency of recessive alleles in sampling sites from the frequency of recessive homozygotes. Cfit-7 uses a simulated annealing algorithm to simultaneously fit clines allowing comparison of their slopes (concordance) and positions (coincidence). When using Cfit, I replicated every run using 10 sets of random seeds. As a first step, I tested whether the clines were best described by simple sigmoidal models (two parameters: centre and slope) or asymmetric stepped models (a sigmoid with an exponential tail to the right; four parameters: centre, slope, distance from centre that tail starts, slope of tail), and used AIC to rank the candidate models.

I then tested the null hypothesis that cline shapes and positions are unchanged between the 1986 and 2011 using i) symmetric models and ii) asymmetric models. i) For each locus, I fitted clines to the two time periods specifying a single centre and slope for both (two parameters). I then compared the likelihood of this null model to the likelihood of models simultaneously fitted with different centres (three parameters) or different slopes (three parameters). ii) Mallet et al. (1990) showed that because of dominance colour pattern clines are not symmetric, and that because of linkage disequilibrium even the codominant *D* locus in *H. erato*, and the *B* locus in *H. melpomene* (dominant in the Andes) exhibit cline shapes similar to the other,

dominant loci. I therefore investigated whether fitting asymmetric clines (generated by adding an exponential tail which can approximate the effect of dominance) altered the conclusions drawn from the symmetric models. For each locus, I again started by testing the null hypothesis that a single cline adequately describes both time periods, but here I specified a single centre, slope and tail position and tail slope (four parameters). The tail was constrained to start within 2.5km of the centre of the cline, and the tail slope was allowed to vary from 0-2.5. These constraints were necessary to generate models with realistic approximations of dominance, where the start of asymmetrical, non-sigmoidal side begins close to the centre of the cline. I then compared the likelihood of this null model with models fitted with different centres (five parameters) and different slopes (five parameters).

Finally I tested for asymmetrical widening of the clines (expected from climate change or habitat alteration) by comparing the slopes of tails fitted to the clines. I fitted right or left tails to both datasets (four parameters), and then compared the likelihoods to models where a different tail slope was fitted to each time period (five parameters). The tails were again constrained to start between 0-2.5km of cline centres, but the slopes were free to take any value.

The null and alternate models were compared using likelihood ratio tests, where the test statistic D is calculated as $2(\text{log-likelihood}_A - \text{log-Likelihood}_0)$. D follows a χ^2 distribution and was tested for statistical significance, with the degrees of freedom for the test given as the difference in the number of parameters being estimated under the alternate and null models. In total, we performed 42 likelihood ratio tests, so I applied a Bonferroni correction to adjust the critical significance levels of α and report results where 5% significance is accepted when $p \leq 0.00119$.

Correlations with climate

For *H. erato* I tested whether the position of the hybrid zone is associated with climate using two datasets: 1) a local-scale Peruvian dataset comprising Mallet et al.'s (1990) data combined with that presented here, and 2) the continent-wide database of museum records presented in Chapter 2. *H. erato* was used rather than *H. melpomene* because many more samples were available. I obtained 19 gridded climate variables from the Worldclim website (http://www.worldclim.org). These were annual mean temperature, mean diurnal range, isothermality, max temperature of warmest month, min temperature of coldest month, temperature annual range, mean temperature of wettest quarter, mean temperature of driest quarter, mean temperature of warmest quarter, mean temperature of coldest quarter, annual precipitation, precipitation of wettest month, precipitation of driest month, precipitation seasonality, precipitation of wettest quarter, precipitation of driest quarter, precipitation of warmest quarter, and precipitation of coldest quarter. I used a generalised linear model (glm) with binomial errors and logit link to model the relationship between the proportion of hybrids and climatic variables and altitude. For the local-scale dataset, I created a 30 arc-second grid aligned with the climate data, and pooled Mallet el al.'s (1990) data and the present data into the cells of the grid, with the cells then comprising sample units. Because a binomial response covers probabilities in the range 0-1, it seemed unsuitable for use with heterozygotes at a given locus in the Peruvian dataset which should not surpass a frequency of 0.5. Because of dominance, diagnosing specimens as hybrids based on phenotypes would also not result in estimates of hybrid proportions that approach 1. Using phenotypic hybrids would also give an asymmetrical estimation of the climatic conditions of the hybrid zone, again because of dominance. I therefore used the allele frequencies estimated above to generate the

expected number of pure and hybrid specimens at each site, where pure specimens are those with either an Andean or Amazonian genotype. Thus following Hardy-Weinberg principles, in the centre of the hybrid zone (where allele frequencies for three loci are ~0.5) the expected proportion of individuals that will be heterozygous at one or more of the three loci is so high as to approach 1 (i.e. expected proportion of specimens that are either pure Andean or Amazonian = $2 \times 0.25^3 = 0.03$). The proportion of pure specimens at a site was therefore estimated as the observed proportion of homozygotes at the *D* locus x the estimated proportion of homozygotes at the *C* locus x the estimated proportion of homozygotes at the *S* locus. Because the glm takes into account sample sizes the resulting proportion was then converted to the estimated number of pure specimens in the sample. For the continental dataset, I created a 10km x 10km equal area grid and pooled the museum specimens found within each grid cell to form sample units. I then took the coordinates of the sample unit as the median latitude and longitude values from the collecting sites comprising the sample unit.

The explanatory variables were standardised so as to get comparable coefficients independent of the measurement unit by subtracting the mean from each variable's values and dividing the result by the standard deviation. Generalised variance-inflation factors (VIFs) were used to assess multicollinearity among the explanatory variables. I applied backward selection to identify the maximum set of explanatory variables where all VIFs were less than 3 (i.e. I fitted a model with all explanatory variables and removed the variable with the highest VIF value, before refitting and recalculating VIF values). I used analysis of deviance to determine which terms to retain in the resulting model. To validate the model I plotted the Pearson's residuals against fitted values and all explanatory variables, and tested them for spatial

autocorrelation (which can violate the assumption of independence of errors) using Mantel tests and Mantel correlograms (using Pearson's correlation coefficient). For both datasets, I then mapped the models predictions using the Worldclim gridded data. In addition, I combined the specimens collected in 1990 and 2011 with a database of geographical records (Chapter 2) and present maps of the hybrid zones between *H. erato favorinus* and *H. melpomene amaryllis* and other *H. erato* and *H. melpomene* races and semispecies (figures 4.5A and 4.5B). When mapping the hybrid zones I included only modern, reliably georeferenced data.

Results

Spatial and temporal change

In 2011 I collected 438 *H. erato* and 466 *H. melpomene*. Table 4.1 compares the likelihoods of symmetric and asymmetric models for each locus within the two time periods. For *H. erato*, all loci in both time periods were best described by an asymmetric cline (i.e. fitted with an exponential tail on the right). For *H. melpomene*, all loci in the 1990 dataset were also best described by asymmetric clines, as were the *D* and *Y* loci in the 2011 dataset. However, the *B* and *N* loci in 2011 were best described by symmetric sigmoidal models.

When comparing the centres and slopes of symmetric sigmoidal clines (table 4.2A, figure 4.2), the *H. erato S* locus and the *H. melpomene B* locus showed a significant shift in the centre towards the Andes, and the *H. erato C*, *H. erato S* and *H. melpomene N* loci showed significant increases in the slopes of the clines. However, only the changes in the *H. erato S* locus remained significant after Bonferroni correction. When comparing the centres and slopes of asymmetric clines (table 4.2B), both the *H. erato S* locus and *H. melpomene B* locus showed significant shifts towards

the Andes. Although these p values were marginally non-significant after a Bonferroni correction, both p-values were extremely low (*H. melpomene B*: p = 0.004492, *H. erato S*: p = 0.001191, critical value p ≤ 0.00119). The *H. erato S* locus also showed an increase in the cline slope, where as the *H. melpomene B* locus showed a decrease in cline slope (both significant after Bonferroni correction).

The *H. erato D*, *H. erato S* and *H. melpomene B* loci all showed significant reductions in the slope of tails on the Andean side of the clines (table 4.3A, figure 4.3), but only the change in the *B* locus was significant after Bonferroni correction. The *H. erato S*, *H. melpomene B* and *H. melpomene N* loci all showed significant increases in the slope of tails on the Amazonian side of the cline (table 4.3B, figure 4.3). The change in *H. erato S* remained significant after Bonferroni correction, and the change in *H. melpomene B* was marginally non-significant (p = 0.00128).

Clines that are the result of secondary contact between neutral traits are expected to decay (Endler 1977). The width of the cline t generations after the populations met in an abrupt step is $2.51\sigma\sqrt{t}$, where σ is dispersal measured as the standard deviation of the distance between parent and offspring (Barton and Gale 1993). Assuming σ to be 2-3km, with 4 generations per year and a initial width of 8.5-13.4km in 1986 (depending on the locus) (Mallet et al. 1990), if the colour pattern clines were the product of neutral mixing following secondary contact they would be expected to have widened to between 51– 76km by 2011. Therefore, that the widths of the clines did not increase symmetrically over the time period is strong evidence that the colour patterns are not neutral traits.

Tables 4.1. Maximum likelihoods estimates for symmetric and asymmetric clines.

locus	model	log likelihood	centre	slope	tail position	tail slope	AIC	Δi
erato C 1990	symmetric	-1054.64	124.86	-0.26			2113.27	122.23
	asymmetric	-992.52	123.76	-0.45	1.89	0.23	1991.04	0.00
erato D 1990	symmetric	-586.69	124.15	-0.41			1177.39	20.37
	asymmetric	-575.51	123.91	-0.52	1.93	0.61	1157.02	0.00
erato S 1990	symmetric	-1061.79	125.24	-0.26			2127.58	128.99
	asymmetric	-996.30	124.05	-0.47	1.49	0.25	1998.59	0.00
erato C 2011	symmetric	-204.90	124.63	-0.31			413.80	34.08
	asymmetric	-186.86	122.58	-0.74	1.42	0.12	379.71	0.00
erato D 2011	symmetric	-143.92	124.06	-0.36			291.85	0.34
	asymmetric	-142.75	124.55	-0.33	0.00	1.81	291.51	0.00
erato S 2011	symmetric	-145.61	123.08	-0.42			295.21	1.73
	asymmetric	-143.74	123.59	-0.38	0.00	1.99	293.48	0.00
melp. B 1990	symmetric	-507.55	123.06	-0.30			1019.11	93.80
	asymmetric	-459.66	120.14	-1.26	0.03	0.15	925.31	0.00
melp. D 1990	symmetric	-496.32	122.38	-0.31			996.63	12.30
	asymmetric	-489.17	121.54	-0.40	1.84	0.48	984.34	0.00
melp. N 1990	symmetric	-498.56	123.15	-0.31			1001.13	19.02
	asymmetric	-488.05	121.26	-0.52	0.00	0.46	982.11	0.00
melp. Y 1990	symmetric	-490.32	123.63	-0.32			984.64	7.44
	asymmetric	-485.60	122.26	-0.44	0.00	0.61	977.20	0.00
melp. B 2011	symmetric	-187.53	121.68	-0.35			379.06	0.00
	asymmetric	-187.15	121.49	-0.36	0.51	1.22	380.30	1.25
melp. D 2011	symmetric	-176.15	124.08	-0.31			356.30	3.77
	asymmetric	-173.27	122.82	-0.37	1.84	0.39	352.54	0.00
melp. N 2011	symmetric	-137.74	123.04	-0.39			279.49	0.00
	asymmetric	-137.61	122.52	-0.43	0.43	1.00	281.21	1.72
melp. Y 2011	symmetric	-160.37	124.56	-0.32			324.73	5.45
	asymmetric	-156.64	122.77	-0.43	0.39	0.48	319.28	0.00

Table 4.2. Likelihoods and estimated centres and slopes for null and alternate symmetric (2A) and asymmetric (2B) models. Likelihood ratio tests significant at the 5% level are shown in yellow, those that remained significant following Bonferroni correction are show in red.

Table 4.2A.

Model	Parameter	*erato C*	*erato D*	*erato S*	*melp. B*	*melp. D*	*melp. N*	*melp. Y*
null	**log likelihood**	-1262.48	-731.57	-1235.72	-698.61	-992.63	-639.74	-652.37
	centre	124.85	124.11	124.90	122.78	122.38	123.21	123.84
	slope	-0.27	-0.39	-0.28	-0.31	-0.31	-0.34	-0.32
alternate	**log likelihood**	-1262.32	-731.57	-1223.26	-696.24	-992.63	-639.60	-650.71
	centre 1990	124.90	124.11	125.34	123.05	122.38	123.14	123.63
	centre 2011	124.63	124.11	122.96	121.94	122.38	123.41	124.59
	slope	-0.27	-0.39	-0.29	-0.32	-0.31	-0.34	-0.32
	D	0.31	0.00	24.90	4.74	0.00	0.28	3.32
	P	0.58	1.00	0.00	0.03	1.00	0.60	0.07
alternate	**log likelihood**	-1259.66	-730.64	-1220.91	-698.42	-992.63	-636.33	-652.11
	slope 1990	-0.26	-0.40	-0.25	-0.30	-0.31	-0.31	-0.32
	slope 2011	-0.31	-0.36	-0.41	-0.32	-0.31	-0.39	-0.34
	centre	124.81	124.14	124.66	122.74	122.38	123.12	123.83
	D	5.63	1.85	29.61	0.38	0.00	6.80	0.52
	P	0.02	0.17	0.00	0.54	1.00	0.01	0.47

Table 4.2B.

Model	Parameter	*erato C*	*erato D*	*erato S*	*melp. B*	*melp. D*	*melp. N*	*melp. Y*
null	**log likelihood**	-1183.54	-723.07	-1167.41	-669.59	-978.33	-629.58	-642.92
	centre	123.45	123.88	124.02	121.19	121.53	121.60	122.38
	slope	-0.49	-0.46	-0.43	-0.46	-0.40	-0.48	-0.44
	x pos	1.59	2.32	2.28	1.28	1.85	0.00	0.00
	x slope	0.23	0.70	0.25	0.32	0.48	0.57	0.60
alternate	**log likelihood**	-1182.43	-723.01	-1162.15	-665.55	-978.33	-629.20	-642.41
	centre 1990	123.71	123.91	124.37	121.20	121.53	121.50	122.26
	centre 2011	123.17	123.77	122.59	119.39	121.53	121.91	122.77
	slope	-0.47	-0.46	-0.44	-0.53	-0.40	-0.48	-0.43
	x pos	1.83	2.31	2.07	0.00	1.85	0.00	0.00
	x slope	0.22	0.69	0.29	0.45	0.48	0.57	0.60
	D	2.23	0.12	10.50	8.07	0.00	0.77	1.02
	P	0.14	0.73	0.00	0.00	0.99	0.38	0.31
alternate	**log likelihood**	-1184.98	-721.53	-1159.89	-664.08	-978.33	-628.79	-642.92
	centre	122.97	123.90	123.91	120.92	121.53	121.69	122.38
	slope 1990	-0.51	-0.48	-0.39	-0.65	-0.40	-0.45	-0.44
	slope 2011	-0.64	-0.41	-0.57	-0.42	-0.40	-0.51	-0.44
	x pos	0.87	2.10	2.47	0.22	1.85	0.00	0.00
	x slope	0.28	0.70	0.26	0.33	0.48	0.61	0.60
	D	-2.89	3.07	15.04	11.01	0.00	1.59	0.00
	P	n/a	0.08	0.00	0.00	0.99	0.21	1.00

Table 4.3. Likelihoods and estimated parameter values for null and alternate models when comparing the slope of fitted exponential tails on left (3A) and right (3B) sides of the cline. Likelihood ratio tests significant at the 5% level are shown in yellow, those that remained significant following Bonferroni correction are show in red.

Table 4.3A

Model	Parameter	*erato C*	*erato D*	*erato S*	*melp. B*	*melp. D*	*melp. N*	*melp. Y*
null	**log likelihood**	-1192.53	-725.20	-1184.03	-668.73	-975.59	-624.55	-640.54
	centre	122.14	123.41	122.48	120.79	121.20	121.63	122.37
	slope	-0.15	-0.33	-0.16	-0.18	-0.23	-0.21	-0.20
	x pos	0.00	0.00	0.00	0.00	0.00	0.49	0.84
	x slope	6.66	2.33	5.46	4.53	2.87	3.77	3.59
alternate	**log likelihood**	-1191.64	-722.26	-1181.34	-654.11	-975.59	-624.52	-640.45
	centre	122.11	123.38	122.35	120.16	121.20	121.88	122.32
	slope	-0.15	-0.33	-0.16	-0.16	-0.23	-0.22	-0.20
	x pos	0.00	0.00	0.00	0.00	0.00	0.77	0.73
	x slope 1990	6.36	2.70	6.57	11.68	2.87	3.37	3.54
	x slope 2011	8.56	1.89	4.47	4.22	2.87	3.55	3.77
	D	1.78	5.87	5.37	29.24	0.00	0.06	0.19
	P	0.18	0.02	0.02	0.00	1.00	0.80	0.66

Table 4.3B

Model	Parameter	*erato C*	*erato D*	*erato S*	*melp. B*	*melp. D*	*melp. N*	*melp. Y*
null	**log likelihood**	-1183.54	-723.07	-1167.41	-669.59	-978.33	-629.58	-642.92
	centre	123.45	123.88	124.02	121.19	121.53	121.60	122.38
	slope	-0.49	-0.46	-0.43	-0.46	-0.40	-0.48	-0.44
	x pos	1.59	2.32	2.28	1.28	1.85	0.00	0.00
	x slope	0.23	0.70	0.25	0.32	0.48	0.57	0.60
alternate	**log likelihood**	-1182.82	-723.05	-1144.04	-664.40	-978.33	-627.48	-642.55
	centre	123.45	123.88	123.78	121.19	121.54	121.60	122.39
	slope	-0.49	-0.46	-0.45	-0.46	-0.40	-0.48	-0.44
	x pos	1.58	2.30	1.34	1.11	1.84	0.00	0.00
	x slope 1990	0.22	0.69	0.28	0.29	0.48	0.52	0.63
	x slope 2011	0.30	0.73	1.41	0.77	0.48	0.82	0.50
	D	1.44	0.04	46.73	10.38	0.00	4.20	0.73
	P	0.23	0.84	0.00	0.00	0.99	0.04	0.39

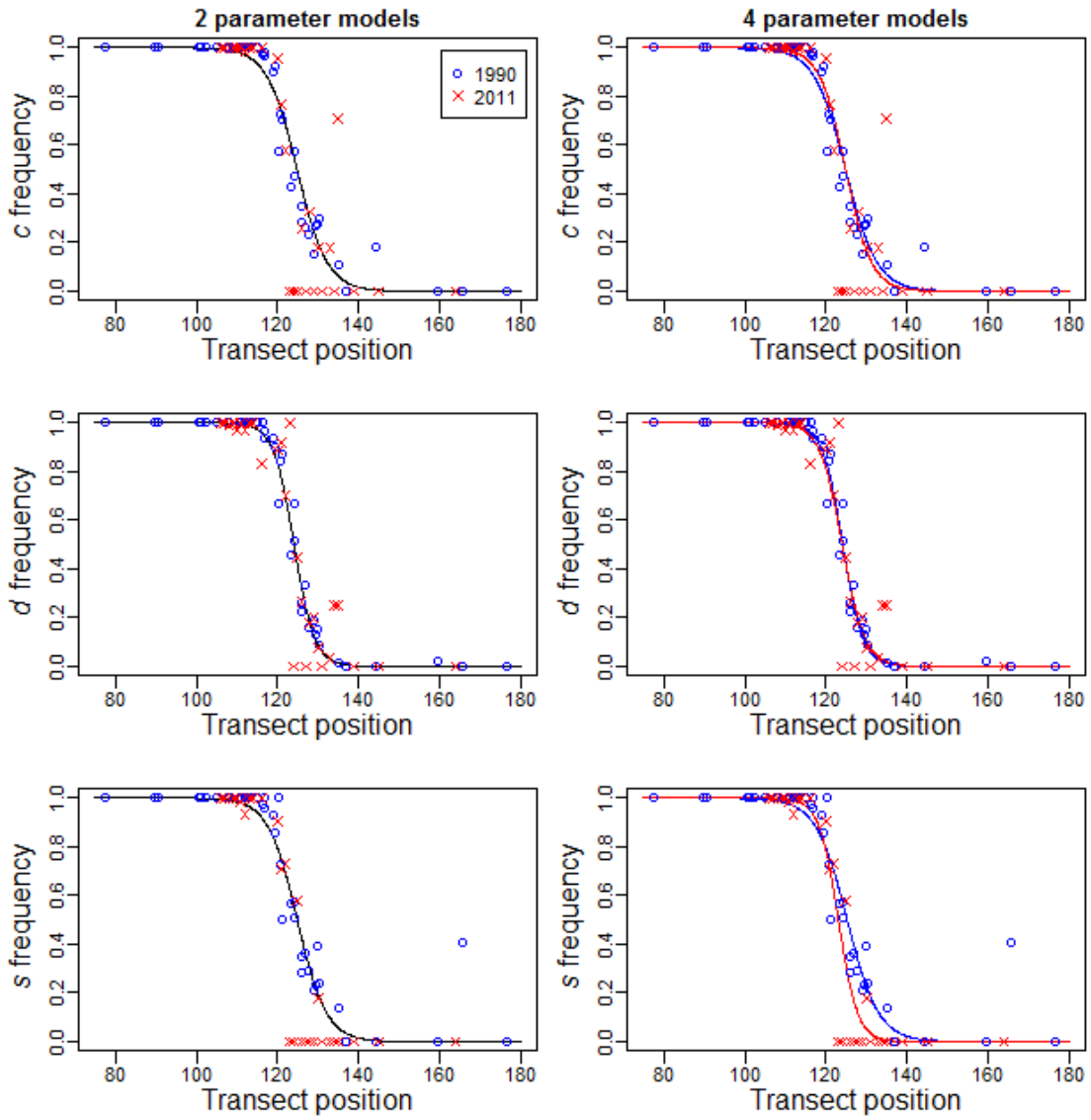


Figure 4.2A. For caption see next page.

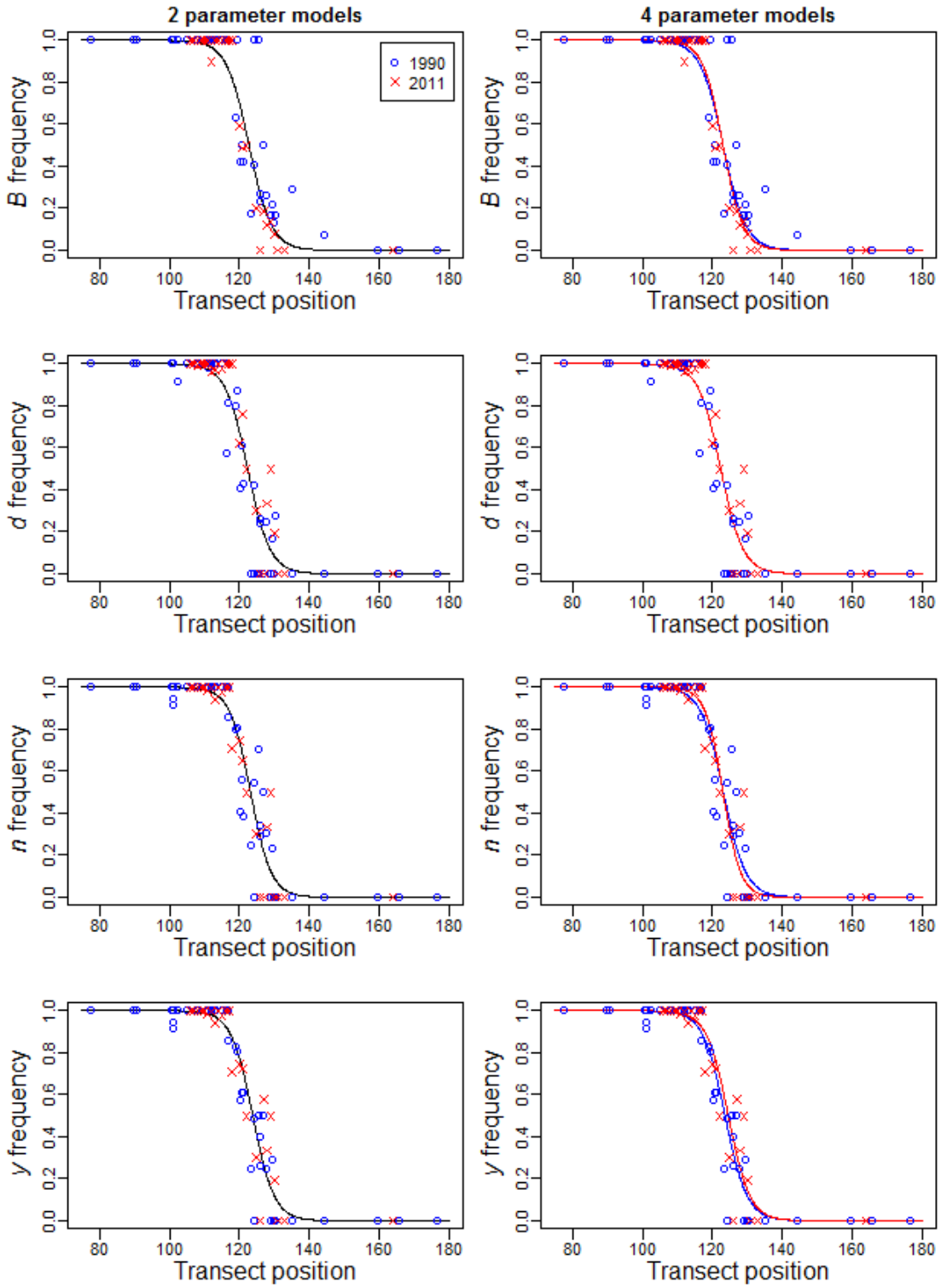


Figure 4.2. Symmetrical colour pattern clines for *H. erato* (4.2A; previous page) and *H. melpomene* (4.2B; above). Left hand column: 1990 and 2011 datasets are fitted with a single cline (in black). Right hand column: 1990 and 2011 datasets are simultaneously fitted with clines specifying a slope and centre for each data set (in blue and red respectively).

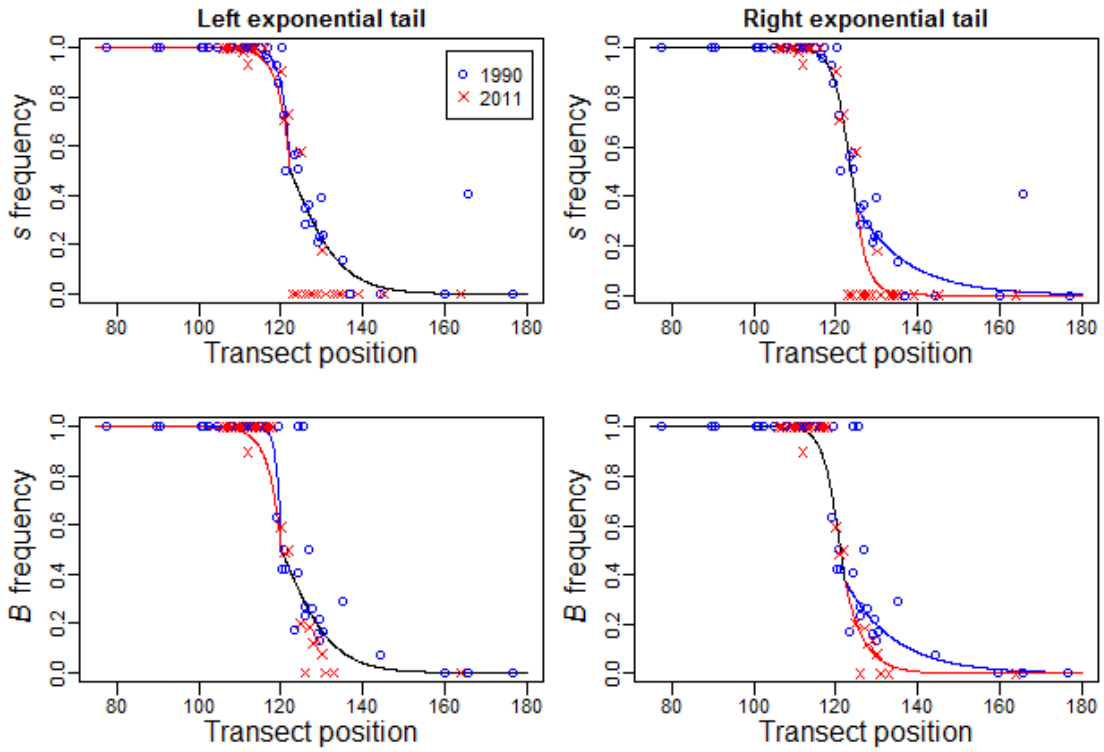


Figure 4.3. Asymmetry of changes in cline shape for the *S* locus in *H. erato* (left) and the *B* locus in *H. melpomene* (right).

Correlations with climate – local-scale data

Backward selection using VIFs resulted in a model with mean diurnal temperature range, temperature seasonality, mean temperature of the warmest quarter and precipitation of the driest month as explanatory variables. I detected overdispersion in the model and so corrected the standard errors using a quasi-GLM model where the variance is given by ϕ x μ, where μ is the mean and ϕ is the dispersion parameter. Plots of the Pearson's residuals (scaled to account for overdispersion) against fitted values revealed one notable outlier: km17 Yurimaguas to Tarapoto in Mallet el al.'s (1990) dataset (km 165.78 on transect). Here a single recessive homozygote (*ss*) in a small sample (six individuals) resulted in a high expected frequency of hybrids for a site far into the Amazon. Removal of this site from the analysis considerably reduced the residual deviance but had little effect on parameter estimation and I present those results here. No clear patterns between the Pearson's residuals and the explanatory variables were discernable. A Mantel test applied to the scaled Pearson residuals found no significant spatial autocorrelation (10000 permutations, r = -0.09, p = 0.93). The Mantel correlogram showed significant but very weak positive spatial autocorrelation between sites at intermediate distance classes (figure 4A; distance classes 60-65, 70-75 and 75-80km). All explanatory variables in the model were highly significant, and analysis of deviance (F test) showed that all terms made significant contributions to the deviance explained (table 4.4). I therefore retained all the explanatory variables in the final model, which explained 79% of the variation in hybrid frequencies. Mean diurnal temperature range and precipitation of the driest month were positively related to the proportion of hybrid specimens, and temperature seasonality, mean temperature of the warmest quarter were negatively related. Precipitation of the driest month explained by far the most variation of the four

explanatory variables. A map of the spatial predictions of the model is presented in figure 4.6. The model predicts the location of other known hybrid zones between *H.erato favorinus* and *H. erato emma* reasonably well. Interestingly, the position of the hybrid zone between *H. erato favorinus* and *H. himera* in the vicinity of Rodriguez de Mendoza is also reasonably well predicted. To validate the environmental data I obtained rainfall data from AGTECA (Agrotechnologica Amazonica; http://www.agteca.com) (figure 4.8). These data comprised historical averages for varying time periods, depending on the particular weather station. While the gridded Worldclim data accorded well with the AGTECA data for Chazuta (Worldclim annual precipitation= 1487mm vs. AGTECA = 1494mm, 18 years), Shanusi (2193mm vs. 2130mm, 16 years), Tarapoto (1168mm vs. 1144mm, 53 years), Yurimaguas (2099mm vs. 2106mm, 53 years), the rainfall in Pongo del Caynarachi at the centre of the hybrid zone was underestimated (2405mm vs 3252mm, 18 years). Other sites close to the hybrid zone (e.g. Lamas, Navarro, Pelejo, Sauce, Shucshuyacu, San Antonio de Cumbaza) also accorded well with the gridded dataset.

Correlations with climate – continental-scale data

Backward selection using VIFs resulted in a model with mean diurnal temperature range, temperature seasonality, mean temperature of the wettest quarter, precipitation seasonality, precipitation of the warmest quarter and precipitation of coldest quarter as explanatory variables. I detected overdispersion and so applied a quasi-GLM model as above. Analysis of deviance indicated that mean diurnal temperature range and precipitation of the warmest quarter did not make significant contributions to the explained deviance, and these terms were removed from the model. All remaining terms were significant, and explained 15% of the variation in hybrid frequencies (table 4.5). Proportion of hybrids was negative related to temperature and

precipitation seasonality, and positively related to mean temperature of the wettest quarter and precipitation of the coldest quarter. Precipitation of the coldest quarter explained by far the most deviance of the four explanatory variables. No clear patterns between the scaled Pearson's residuals and the explanatory variables were discernable. A mantel test applied to the scaled Pearson residuals found no significant correlation between and geographic distance (10000 permutations, $r = -0.07$, $p = 1$). The mantel correlogram showed extremely weak but significant spatial autocorrelation at some distance classes (figure 4.4B). The predictions for the model are mapped in figure 4.7. Few areas with are predicted to have a high proportion of hybrids; this is presumably because the sample sites used in the model rarely have a frequency of hybrids >0.5 for the same reasons discussed previously. The Choco region of Colombia stands out as having conditions that are associated with a high frequency of hybrids; this is likely to be because the region is the wettest in South America. The lower Magdalena, the foothills of the eastern Andes around Villavicencio and Mocoa, Southern Venezuela and a number of areas along the course of the Amazon river also had climatic conditions associated with hybrids. Interestingly, the boundary between the south-eastern Brazilian postman races of *H. erato* and the Amazonian dennis-rayed forms coincided well with an increase in the predicted proportions of hybrids, despite no hybrid specimens being recorded from this area.

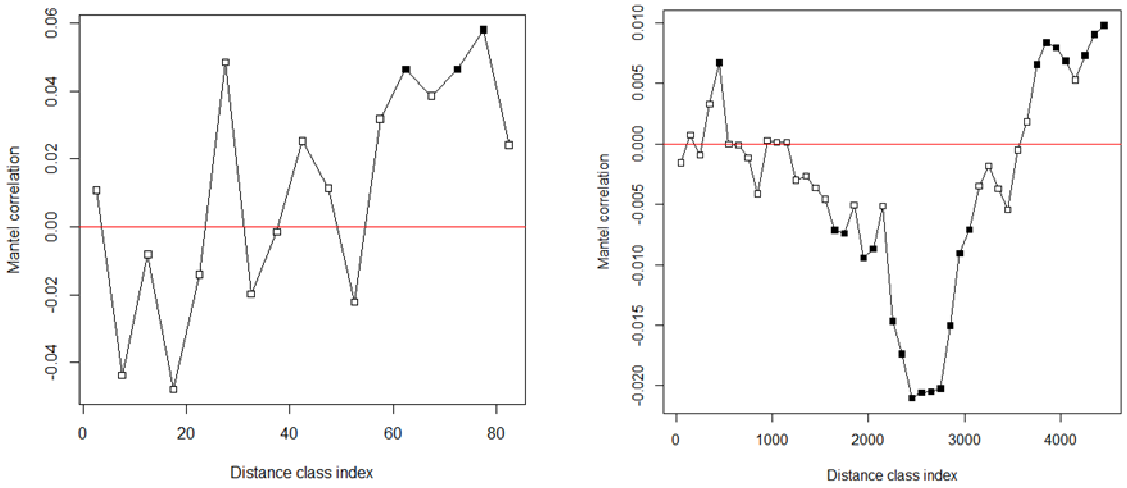


Figure 4.4. Mantel correlograms for local-scale analysis (left; 5km distance class intervals) and continental-scale analysis (right; 100km distance class intervals). Filled squares = statistically significant after Holm correction for multiple tests.

Table 4.4. Model summary and analysis of deviance for local-scale climate analysis.

	Estimate	Std. error	t-value	P-value
Intercept	-1.731	0.172	-10.084	<0.001
Diurnal range	1.734	0.226	7.673	<0.001
Temp. seasonality	-1.444	0.236	-6.122	<0.001
Temp. warmest quarter	-1.546	0.237	-6.533	<0.001
Precip. driest month	1.974	0.228	8.646	<0.001
Dispersion parameter for quasibinomial family taken to be 2.84				
Null deviance: 1430.4 on109 degrees of freedom				
Residual deviance: 302.1 on 105 degrees of freedom				

	DF	Deviance	F	P-value
Residual deviance	-	302.1	-	-
Diurnal range	1	551.7	86.736	<0.001
Temp. seasonality	1	413.7	38.794	<0.001
Temp. warmest quarter	1	426.0	43.051	<0.001
Precip. driest month	1	642.4	118.285	<0.001

Table 4.5. Model summary and analysis of deviance for continental scale climate analysis.

Coefficients	Estimate	Std. error	t-value	P-value
Intercept	-2.083	0.060	-34.894	<0.001
Temp. seasonality	-0.177	0.077	-2.293	<0.05
Temp. wettest quarter	0.490	0.068	7.237	<0.001
Precip. seasonality	-0.234	0.056	-4.196	<0.001
Precip coldest quarter	0.615	0.051	12.167	<0.001
Dispersion parameter for quasibinomial family taken to be 1.92				
Null deviance: 4936.5 on 2464 degrees of freedom				
Residual deviance: 4192.7 on 2460 degrees of freedom				

	DF	Deviance	F	P-value
Residual deviance	-	4192.7	-	-
Temp. seasonality	1	4203.4	6.257	<0.05
Temp. wettest quarter	1	4312.8	70.453	<0.001
Precip. seasonality	1	4227.5	20.404	<0.001
Precip coldest quarter	1	4491.5	175.289	<0.001

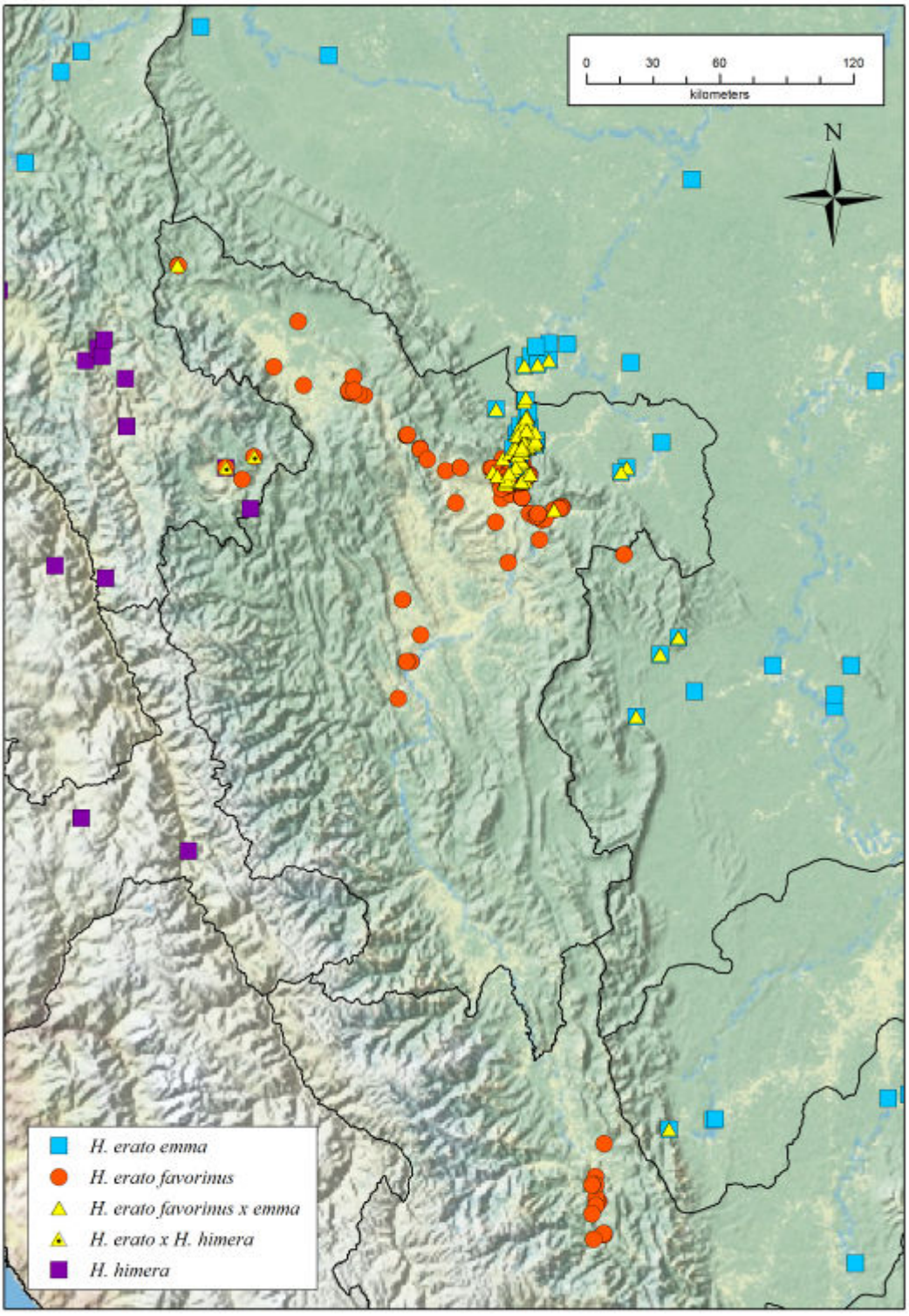
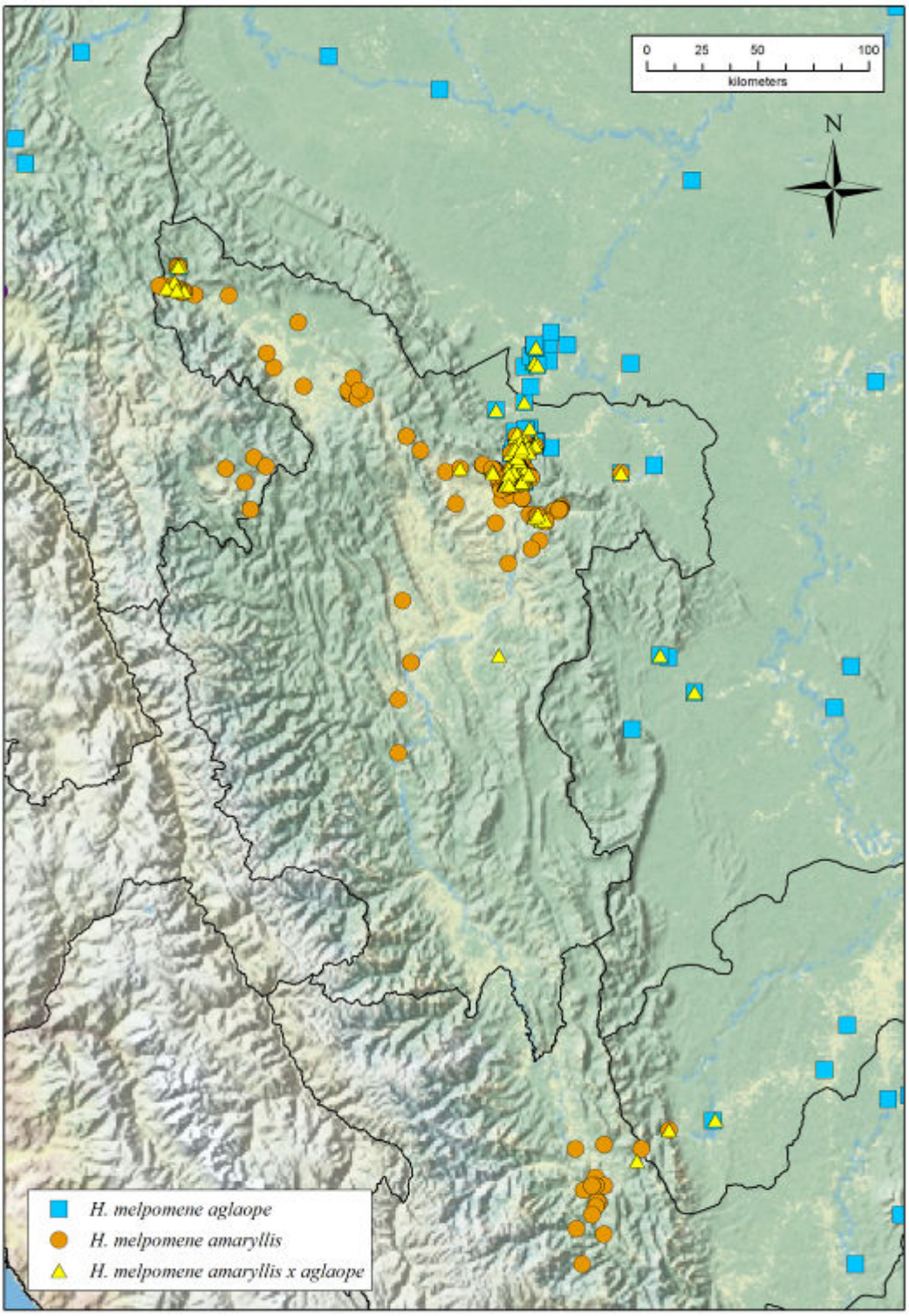

0
30
60
120
kilometers
N
H. erato emma
H. erato favorinus
H. erato favorinus x emma
H. erato x H. himera
H. himera

Figure 4.5A.



Figures 4.5a and 4.5b (directly above) map the hybrid zones that abut the ranges of *H. erato favorinus* and *H. melpomene amaryllis* in the upper Huallaga valley in Peru. Care was taken to include only modern, reliable data in the vicinity of contact zones. In the interests of clarity a handful of specimens identified as dennis-rayed variants (*H. melpomene vicina, H. melpomene malleti, H. erato lativitta*) were lumped with *H. melpomene aglaope* and *H. erato emma* respectively.

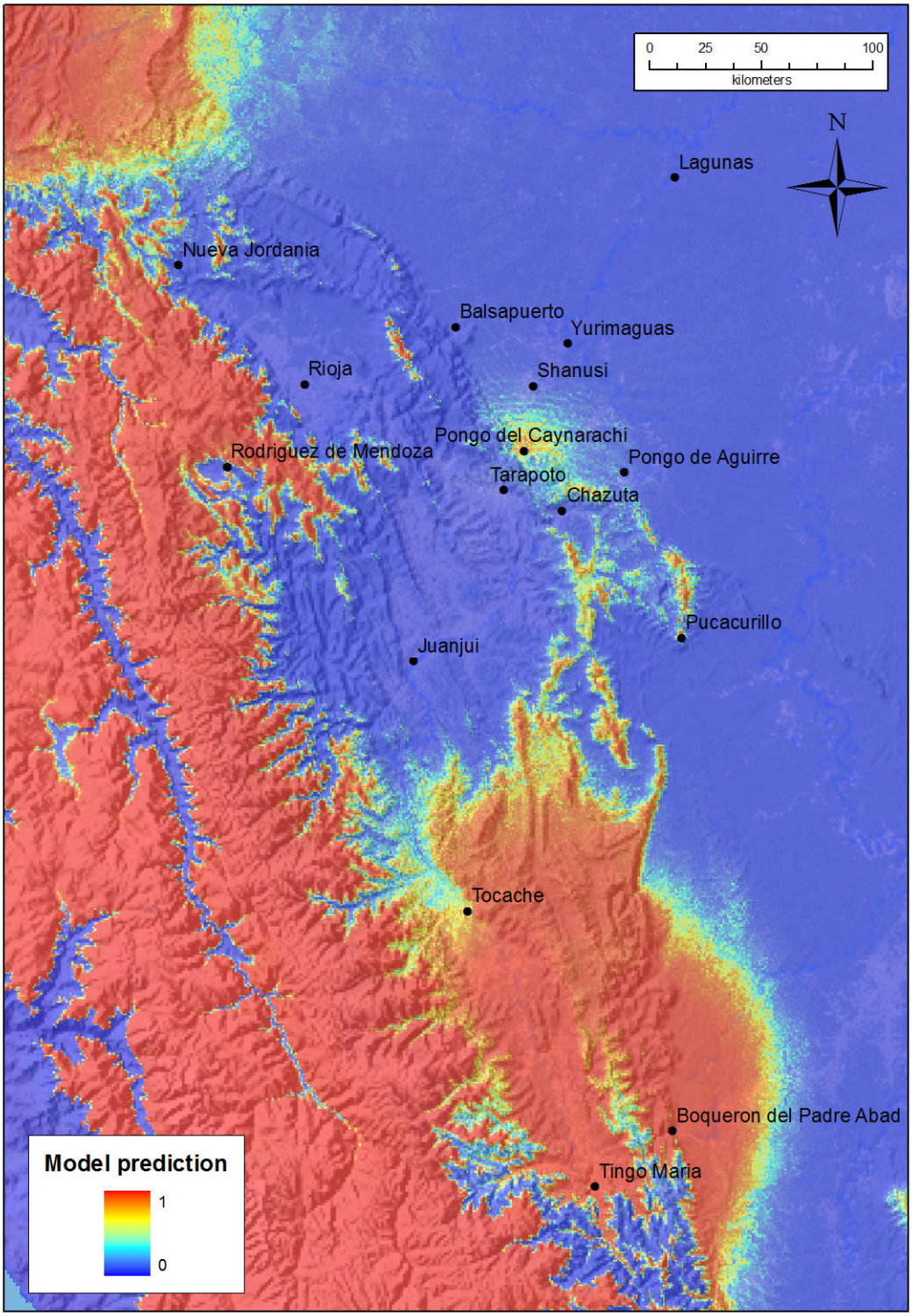


Figure 4.6. Model predictions for hybrid frequencies of *H. erato* in northern Peru.

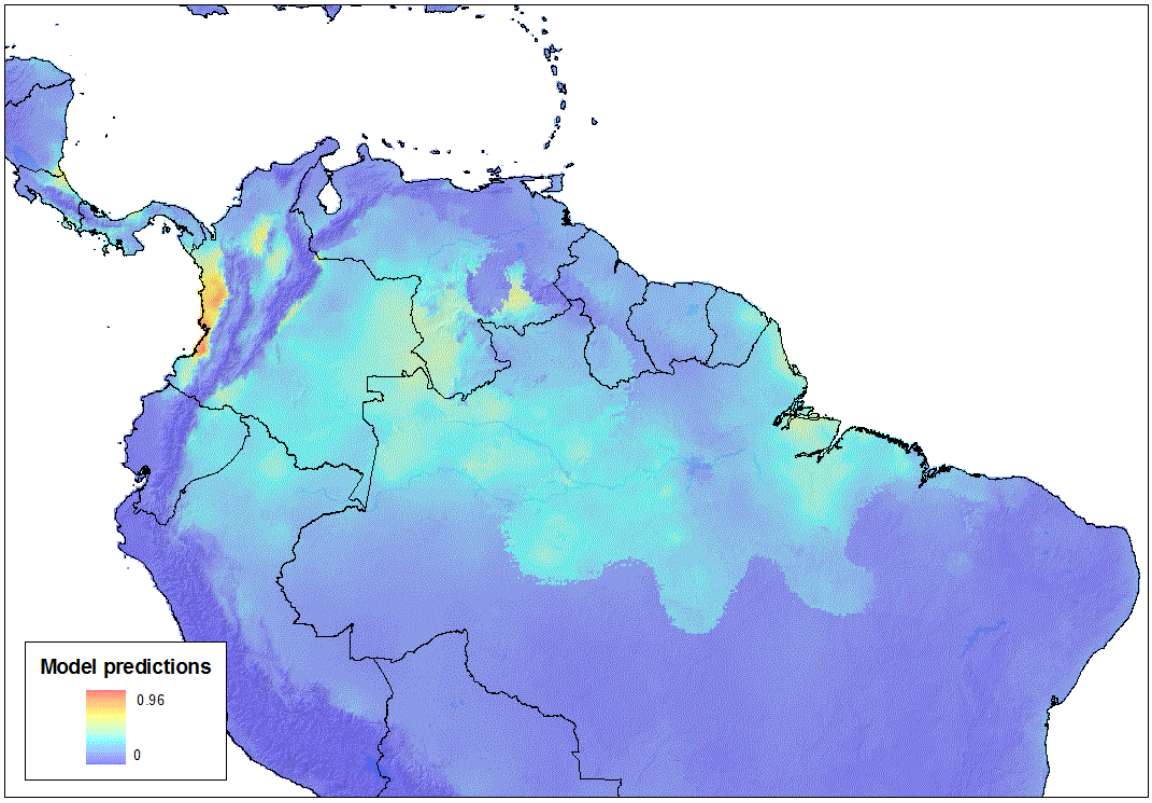


Figure 4.7. Model predictions from the continental-scale *H. erato* data set.

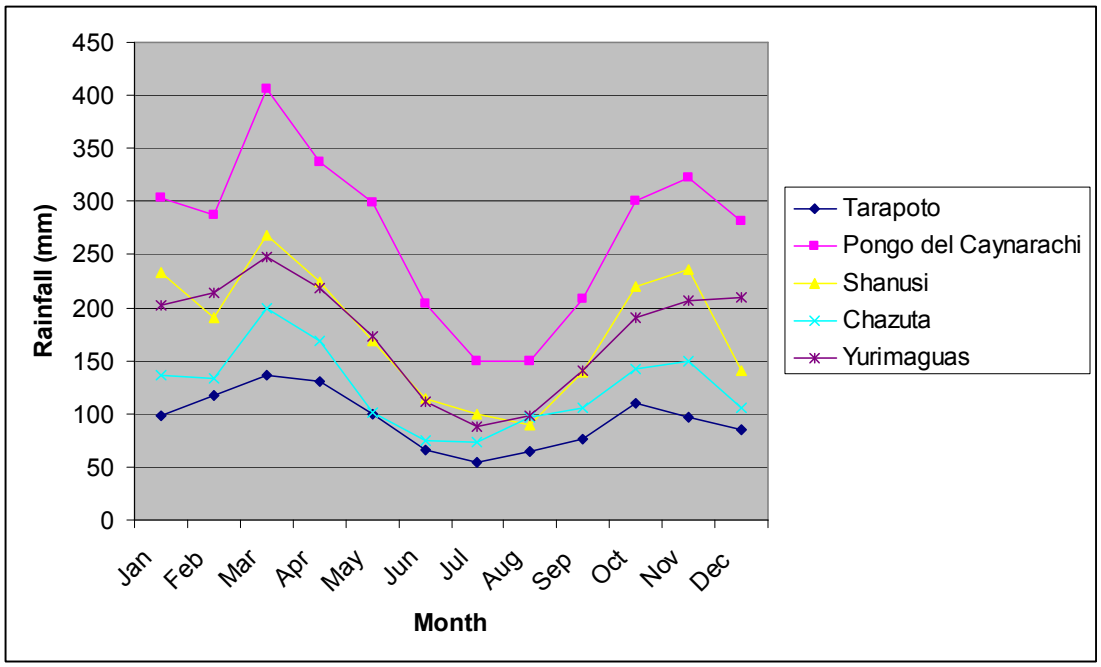


Figure 4.8. Monthly rainfall data (in millimetres) at sites across the hybrid zone. Tarapoto and Chazuta fall on the Andean side of the hybrid zone, Shanusi and Yurimaguas on the Amazonian side and Pongo del Caynarachi falls in the centre of the hybrid zone.

Discussion

Spatial and temporal change

I found that the centre of clines to be remarkably similar between the two time periods. Only the centres of two loci, the *H. erato S* locus and *H. melpomene B* locus were found to have moved significantly, and only by very small distances of about 1-2km. Although both centres had apparently shifted towards the Andes as predicted, the differences in the centres of the clines seem likely to be attributable to asymmetrical changes in the shape of the two clines rather than cline movement. The clines for both loci appear considerably steeper on the Amazonian side of the hybrid zone, and less steep on the Andean side (figure 4.3). There was also evidence for the *H. erato C* and *H. melpomene N* clines being steeper, and for the *H. erato D* locus being somewhat less steep on the Andean side.

None of the colour pattern clines showed evidence of symmetrical widening over the time period, providing strong evidence that they are not the result of neutral mixing following secondary contact. Previous studies using direct evidence from mark-release-recapture experiments that transferred phenotypes across the hybrid zone and indirect, genetic evidence from linkage disequilibrium and cline widths suggested that strong selection against foreign phenotypes maintained the colour pattern clines (Mallet and Barton 1989, Mallet et al. 1990). Therefore, these results add further evidence that selection on colour patterns maintains the clines in *H. erato* and *H. melpomene*.

It is unclear why the *H. erato S* and *H. melpomene B* clines should have become steeper on the Amazonian side of the clines. For the *H. erato S* locus the small samples sizes collected in 2011 may not have been sufficiently large to reliably find

recessive homozygotes, leading to erroneous estimates of zero allele frequencies. However, this does not explain the change in the *H. melpomene B* locus, in which the Amazonian *b* allele is recessive. Perhaps the most likely explanation for the apparent change on the Amazonian side is that the changes simply represent stochastic fluctuations in allele frequencies. Conversely, I did find some evidence for asymmetrical widening of the clines on the Andean side of the hybrid zone. In particular, the frequency of the *H. melpomene b* allele at the mountain pass ("El Tunel"; 6.45S, 76.29W) between the Amazon and upper Huallaga Valley was exceptionally high (10%; 94 specimens) given its position (km 112) on the transect. The frequency of the Amazonian allele *S* in *H. erato* was also high at the km 112 position on the transect (6%; 32 specimens). Unfortunately, Mallet et al. (1990) did not collect from this site in the eighties, thus direct comparisons are not possible. On this side of the hybrid zone the mountains comprise a protected area with deforestation restricted to the valley bottoms, thus a reduction in selection via reduced predator populations seems unlikely to be responsible for a widening of the hybrid zone. Anecdotal evidence suggests that El Tunel was invariably shrouded in mist in the eighties, and that *H. erato* was rare or absent from the area (J. Mallet, pers. obs). While the pass is still one of the first areas to be enveloped in clouds, sunny spells are now frequent and collecting highly productive; both *H. melpomene* and *H. erato* are common (N. Rosser, pers. obs.). An obvious possibility is therefore that a warming trend, possibly caused by deforestation of lowlands to the north-east has reduced cloud cover, and effectively "lowered" the mountain pass, enabling increased gene flow from the lowlands. Indeed, a specimen of *H. melpomene aglaope* was also collected at El Tunel (km 112.06 on the transect) whereas Mallet et al. (1990) did not record *H. melpomene aglaope* until km 46-46.5 Tarapoto-Yurimaguas (km 118.86 on

the transect). An additional factor that may have had some impact is the recent construction in 2010 of high-voltage electric cables from Tarapoto to Pongo del Caynarachi. To allow the cables and pylons to be erected, a clear cut of forest approximately 30 meters wide and running parallel and very close to the transect was made. In the 2011 collecting period, the cut area still comprised low vegetation among which Passiflora spp. were abundant, and it was here that the *H. melpomene aglaope* specimen was collected. It therefore seems possible that the construction works may have allowed an increase in butterfly densities and migration along the transect.

The significant deforestation that has occurred in and around the collecting sites on the Amazonian side of the cline might be expected to have reduced predator populations and consequently selection, leading to a widening of the cline. However, I found no evidence that any of the clines had widened significantly on the Amazonian side. Jacamars (Galbulidae) and Tyrant flycatchers (Tyrannidae) are thought to be the most important visual predators responsible for the frequency dependent selection that maintains the hybrid zone (Chai 1986, 1996, Pinheiro 1997, Bull 2003, Langham 2004). Previous work translocating *H. erato* races across the hybrid zone found increased selection against foreign morphs only at sites where Jacamars were common (Mallet and Barton 1989). It is therefore rather surprising that deforestation does not appear to have affected the hybrid zone, but this may be explained by the fact that Jacamars and especially some species of Tyrant flycatcher adapt well to human altered habitats.

Correlations with climate

The statistical models of the hybrid zone supported the hypothesis that rainfall may be responsible for trapping the tension zone at the foot of the Andes (Mallet 1993), with

precipitation explaining far more of the variation in hybrid proportions that the other climatic variables. In fact, weather station data from El Pongo del Caynarachi averaged across an 18 year period showed that rainfall in the centre of the hybrid zone was greatly underestimated by the gridded data used in the analysis. Even the driest month at the centre of the hybrid zone is wetter than the wettest month in the Tarapoto on the Andean side of the hybrid zone, and also wetter than most months in Yurimaguas on the Amazonian side (figure 4.8). It is easy to see how areas of heavy rainfall could result in a population density trough for butterflies which could trap the hybrid zone. *Heliconius* are most active on sunny days with greatly reduced activity on cloudy days, and rain causes complete inactivity. Thus at best rain prevents population growth, but is also probably physically detrimental to the point that some butterflies are killed.

Although rainfall appears to be the most important determining environmental factor determining the position of the hybrid zone, mean diurnal temperature range was also positively related to the proportion of hybrids and explained a large proportion of the deviance. While an association between diurnal temperature range and the hybrid zone is not immediately obvious from field experience, a positive association seems biologically sensible, because low night time temperatures might well reduce the hours that butterflies can spend active in the daytime. Similarly, the negative association between mean temperature of the warmest quarter and the hybrid zone is intuitive, because average temperatures should likewise act to reduce butterfly activity and should contribute to a density trough. A significant negative association between temperature seasonality was not expected, but this variable explains least of the deviance and varies little across the study area.

The hybrid zones between the Amazonian and Andean races of *H. erato* and *H. melpomene* comprises two of a much larger number of butterfly contact zones that occur together in a well studied "suture zone" (Whinnett et al. 2005, Dasmahapatra et al. 2010). Interestingly, the centres of hybrid zone between other species are not always located at the base of the mountains. The hybrid zone between races of the ithomiine butterfly *Oleria onega*, for example, is thought to occur well into the mountains (Galluser 2002, De-Silva 2010). *Oleria* are typical of closed canopy wet forests, and frequently fly in overcast conditions. Thus, if the *O. onega* hybrid zone is a tension zone, the high precipitation on the North-eastern flanks of the mountains may not be relevant as a density trough and other factors such as temperature maybe more important.

As a test of the statistical model, I mapped its spatial predictions. The positions of other known hybrid zones were reasonably well predicted. Moving away from the study area the model quickly ceases to predict the hybrid zone to be located at the foot of the mountains and instead predicts it to be found at higher elevations (figure 4.6). There is evidence that this prediction is accurate; at Pucacurillo (c. 1000m) in the Parque Nacional Cordillera Azul (110km SE of the hybrid zone), Dasmahapatra et al. (2006) collected 9 specimens of *H. erato*, of which 5 were hybrids and 4 were *H. erato emma*. Although a small sample, the composition is suggestive that the hybrid zone may be indeed be located at higher altitude rather than at the base of the mountains in this area. Another suitable place to test this prediction would at Balsapuerto at the foot of the Andean cordillera some 60km NNE of the hybrid zone transect studied in detail here; here again the model clearly predicts the hybrid zone to be located deeper within the cordillera. In the extreme south of the ranges of the postmen phenotypes, the hybrid zone between postmen and dennis-rayed races is

know to lie approximately at Boqueron del Padre Abad at the base of the Andean cordillera. Here again precipitation peaks at the base of the Andes and the model predicts that the hybrid zone should indeed lie in the lowlands.

In the north of San Martin the location of the *H. melpomene* and *H. erato* hybrid zones is in the Andes in the Alto Mayo watershed. While the exact position of the hybrid zone is again unclear, it appears to be in the vicinity of the hamlet of Nueva Jordania (5.58181°S, 77.67613°W) approximately 60km from the nearest Amazonian lowlands and some 9km north of the Rioja-Pedro Ruiz highway. Here, about 50% of specimens are hybrids (of 46 specimens collected by M. Joron in 2007 and C. Merot in 2011 22 were hybrids, 15 *H. melpomene amaryllis* and 9 *H. melpomene aglaope*). Along the highway, *H. melpomene amaryllis* predominates, but the frequency of hybrid phenotypes seems to be about 10% (from 84 specimens collected from 2002-2011). *H. erato* is rare in the Alto Mayo, but at least one hybrid has also been recorded in Nueva Jordania. Given the differences in habitat and climate to the Tarapoto-Yurimaguas hybrid zone, the presence of the hybrid zone in this area appeared odd. However, inspection of the models predictions suggests this is not surprising; the model indicates that climatic conditions in the foothills should not create a density trough which would trap the cline. Rather the hybrid zone would be expected to sweep into the mountains up to more or less its current position. Mallet et al. (1990) did not record any hybrids from the Alto Mayo, however their collections from this region were few. Given a lack of apparent climatic barriers associated with the current positions of the hybrid zones, the intriguing possibility arises that the hybrid zones in this area may be unstable and free to move. The *H. erato* and *H. melpomene* hybrid zones in the Alto Mayo region are also interesting as they demonstrate that the dennis-rayed phenotype is not just required as an adaptation to

the lowland habitats or climate (e.g. Blum 2008). Similarly, rayed races of *H. erato emma* and *H. melpomene aglaope* penetrate up to ca. 1500m in the Pozuzo Valley further to the south (Mallet 1993). Furthermore, no discrete boundary between habitats is apparent when one crosses the hybrid zone in Pongo del Caynarachi, nor is there any evidence for genomic differentiation between the races other than at colour pattern loci (Baxter et al. 2010, Counterman et al. 2010, Nadeau et al. 2012).

Rather surprisingly, the model also accurately predicted the location of the hybrid zone between *H. himera* and *H. erato favorinus* in the region of Rodriguez de Mendoza (König 1986, Mallet et al. 1990). *H. himera* inhabits dry habitats in the Andes of northern Peru and southern Ecuador and was previously considered a parapatric race of *H. erato*, however recent work has shown a substantial reproductive and genetic discontinuity between the pair (Jiggins et al. 1996). The model also predicts the position of a well studied hybrid zones between *H. himera* and *H. erato* in southern Ecuador, which has previously been interpreted as an example of ecological adaptation and parapatric speciation onto different habitats (Jiggins et al. 1996). However, an alternative explanation is that *H. himera* may represent a relict of previously widely distributed form that has been replaced by more recently evolved races (Chapter 2). If the latter is the case then the model's successful prediction of the hybrid zone's position suggests that the conditions there are consistent with those that are likely to trap a moving hybrid zone. In the *H. himera*/ *H. erato* hybrid zone, rainfall is low and the models predictions that hybrids should occur there are a product of the lower temperatures.

The continental-scale analysis produced similar findings to the local-scale analysis, but the model explained much less of the deviance. Interestingly, however,

precipitation again proved the most important explanatory variable. Although other hybrid zones are not necessarily expected to be associated with regions of high rainfall (e.g. *H. himera*), the association shows that this may be a general pattern. A long standing hypothesis for the origin of new races and species in tropical America is the refugium theory, in which a reduction of forest cover during dry periods in the earth's history induced allopatric speciation in fragmented forest "refugia" where precipitation remained high (Haffer 1969, 2008, Brown 1979). However, the observed correlations between precipitation and hybrids documented here are contrary to the expectations of the refugium theory. In the refugium theory, hybrids are expected to occur in drier areas (between putative wet refuges) compared to pure specimens. The results therefore add to a growing body of work casting doubt on the refugium theory (Nelson et al. 1990; Whinnett et al. 2005; Dasmahapatra et al. 2010; Bush 1994; Endler 1977; Beven et al. 1984; Moritz et al. 2000; Wilf et al. 2003).

Implications for speciation

Previous theoretical and empirical work has demonstrated that clines between newly evolved *Heliconius* races may move across the landscape (Mallet 1986, Blum 2002). Here, a cline predicted to move appears to be trapped and stationary in a zone of exceptionally high rainfall. Other studies of hybrid zones in *H. erato* have concluded that hybrid zones represent ecological adaptation and parapatric speciation to distinct habitats (Jiggins et al. 1996, Blum 2008, Arias et al. 2008). Our results here suggest that subspecies differences, in the case of endogenous selection for mimicry, are not necessarily a reflection of adaptation to the abiotic environment. Instead, hybrid zones may simply be located in regions where environmental conditions reduce population densities and thus trap otherwise mobile hybrid zones. Once a hybrid zone has

become stable in such a region, genetic differentiation and assortative mating may develop.

Conclusions

None of the colour pattern clines showed evidence of widening symmetrically over the time period, suggesting that they are maintained by selection and are not the product of neutral mixing following secondary contact. The data show an apparent increase in the frequency of the *H. erato S* and *H. melpomene b* alleles on the Amazonian side of the tension zone. The reasons for this are unclear, but the high frequencies in the Amazon appear to result in gene flow across the mountain pass separating the Huallaga Valley from the Amazonian lowlands. I found no evidence to support Mallet et al.'s (1990) prediction that the tension zone is moving towards the Andes. Instead, the position of the tension zone seems well established at the base of the Andes, where it is strongly associated with the peak of rainfall. Hybrid specimens of *H. erato* collected across tropical America also show a significant association with rainfall. Therefore, these results run counter to the Pleistocene refugium theory.

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Chapter 5. Conclusions

In this thesis I characterise the geographical and ecological contexts surrounding the evolution of races and species of *Heliconius* butterflies and their allies. Here, I summarise my principal findings and draw further conclusions where possible. Chapter 2 investigates geographical patterns of diversification in the heliconiines. A weak negative correlation between species richness and phylogenetic branch length is consistent with spatial gradients in species richness being driven at least in part by variation in speciation and/or extinction rates, rather than via evolutionary age or niche conservatism alone. The eastern Andes are notable for high species richness and short phylogenetic branch lengths. Conversely, intra-specific phenotypic diversity is highest in the Amazon basin, with a suture zone apparent along the course of the Amazon River. This mismatch suggests differences between the process of subspeciation and speciation. It is proposed that that new colour patterns tend to evolve within the Amazon, but that speciation is more likely to occur in the Andes.

Hines et al. (2011) showed that the geographically disjunct nature of colour patterns observed within *Heliconius erato* could indeed be explained by a new colour pattern originating within the Amazon basin and spreading out, leaving relictual colour pattern races confined to central America, the Andes and south-eastern Brazil. In Chapter 4, I show that the tension zone between these Amazonian / Andean races is stable in a region of peak rainfall. Tension zones are often expected to move due to a variety of reasons (Barton 1979, Mallet 1986); these data demonstrate conditions under which they may stabilise, and thus allow the accumulation of further genetic, ecological, and behavioural differences.

In Chapter 3, simulations of the geography of speciation show that patterns of range overlap observed in heliconiines are consistent with sympatric speciation. Unfortunately, as is frequently the case with such studies that seek to infer process from pattern, the results are open to alternative interpretations. Specifically, parapatric speciation followed by a tendency for species to rapidly to spread into sympatry provides an equally plausible explanation for the observed patterns. If the latter hypothesis is true, then raciation and speciation in heliconiines might be thought to involve two stages. New colour patterns may be seeded in Amazonia, before spreading out and displacing ancestral races. These "waves" will proceed until they reach partial barriers such as the rainfall peak observed in the eastern Andes, at which point they will stabilise and speciation can proceed. Once reproductive isolation and sufficient ecological divergence have developed, species may then expand back into one another's ranges.

I also present evidence in Chapter 3 that shifts in mimetic pattern and host plant shifts are associated with speciation in heliconiines. These results add to a growing body of literature suggesting that mimicry shifts are important in speciation in heliconiines (Jiggins et al. 2001, 2004, Chamberlain et al. 2009, Merrill et al. 2011a, 2011b), and provides the first comparative evidence that host plant shifts may also be important across the Heliconiina. Both results suggest that ecological adaptation may be important in triggering speciation events.

Implications for the evolution of Neotropical biodiversity

In Chapter 1, hypotheses for evolution of Neotropical biodiversity are reviewed. Two of the findings presented in this thesis oppose the Pleistocene refugium theory as the process responsible for generating new races of heliconiines. Firstly, the presence of a

suture zone along the Amazon river is contrary to the expectations of the refugium theory. This is because gallery forest is likely to have persisted along the Amazon even during dry periods, when forest may have been replaced by savannah in other areas. Therefore, contact zones between taxa are not expected to occur along the river itself, rather river populations are expect to be pure (Mallet 1993). Similarly, the correlations between rainfall and hybrid zones presented in chapter 4 are contrary to the refugium theory, in which contact zones are expected to occur in drier regions between wet refugia (Mallet 1993). However, if the contact zones between races that formed after expansion from refugia are mobile, then we might expect them to move until they reach partial barriers to dispersal or density troughs, where they will stabilise and settle (Barton 1979, Mallet 1993). Therefore, our ability to draw conclusions from these data is dependent on the extent to which the current distributions of taxa reflect their distributions when they were formed (Losos and Glor 2003). In contrast, geographic data and mtDNA branch lengths strongly support the importance of the Andes in generating new species of heliconiines (Hoorn et al. 2010). For heliconiines, two plausible explanations are that the Andes present more opportunities either for partial geographic isolation or for ecological speciation (Chapman 1917, Elias et al. 2009).

Historically, hypotheses for the evolution of Neotropical biodiversity have usually been discussed within the framework of allopatric speciation (Haffer 1969). It is apparent from Chapter 2 and Chapter 3 that the only obviously complete barrier to dispersal of heliconiines is the Andean mountain range, and that almost all sister species pairs exhibit at least some range overlap. Furthermore, the patterns of range overlap presented in Chapter 3 are consistent with sympatric speciation. Therefore, these geographic data, combined with data on host plants and mimicry shifts, suggest

that ecological speciation in the face of gene flow, whether parapatric or sympatric, seems a more plausible hypothesis for heliconiines than allopatric speciation.

While phytophagous insects comprise a large portion of biodiversity, mimicry is less common, and it is unclear how many other taxa exhibit the genetic architecture of *Heliconius* that is thought to facilitate speciation with gene flow. Therefore, whether the patterns of diversification observed in heliconiines are typical of other taxa will require biogeographic studies incorporating phylogenetic data to be conducted for a broader range of taxa than have been considered to date. Even in the closely related ithomiine butterflies switches in mimetic pattern are less obviously associated with speciation events, and there appears to be less sympatry between sister species (Jiggins et al. 2006). Sister species of birds are almost invariably allopatric (figure 5.1; Phillimore et al. 2008). However, it is possible that the marked differences in the patterns of range overlap are the product of different taxonomic practises adopted by lepidopterists and ornithologists (with the latter group more inclined to split geographic forms into species), rather than differences in evolutionary processes.

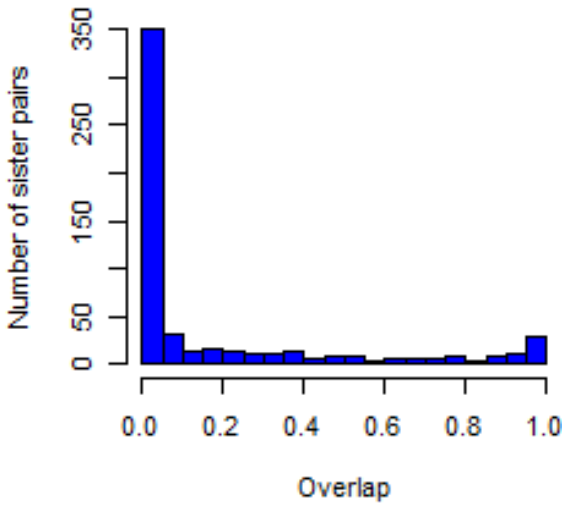

Figure 5.1. Range overlap between sister species of birds. From Phillimore et al. (2008).

Future directions

Two questions stand out as particularly pressing for future research into the speciation and biogeography of heliconiines. Firstly, further evidence from individual case studies is now required to add further support to the likelihood of sympatric speciation occurring in the group. Unfortunately, demonstrating sympatric speciation within a continent seems likely to be difficult (Coyne and Price 2000, Coyne and Orr 2004, Fitzpatrick and Turelli 2006). To build the case for the likelihood of sympatric speciation occurring in heliconiines, it will be necessary to demonstrate points along the speciation continuum, from local polymorphs to sympatric species exhibiting near complete assortative mating. Although heliconiines are typically locally monomorphic, polymorphisms do exist (Joron et al. 1999, Mallet 1999), and in one case have been shown to exhibit weak assortative mating (Chamberlain et al. 2009). In addition, sympatric species with strong, but incomplete assortative mating are also known; in one population of the largely sympatric species pair *H. cydno* and *H. melpomene* up to 8% of individuals are hybrids (Mavárez et al. 2006, Giraldo et al. 2008). Thus, much rests on identifying sympatric "semi-species" exhibiting intermediate levels of assortative mating similar to that observed between the largely parapatric pair *Heliconius erato* and *Heliconius himera* (Jiggins et al. 1996).

It may also be possible to test the hypothesis that rapid range expansion explains sympatry between sister species rather than sympatric speciation. In Chapter 3, it can be seen that from the histograms of range overlap between sister species of heliconiines that sister species are usually sympatric or parapatric; sister species with intermediate levels of range overlap are comparatively rare. These species pairs could potentially be informative with respect to the rapid range expansion hypothesis. If the hypothesis is true and accounts for the high levels of sympatry observed in heliconiine

sister species, then we might expect those with intermediate levels of range overlap to have diverged sufficiently ecologically to allow coexistence, and now be expanding rapidly into one another's ranges.

Secondly, a better understanding of how new colour patterns arise and spread in heliconiines represents a high priority for future research into heliconiine biogeography. Hines et al. (2011) provided the first empirical evidence supporting the hypothesis that new colour pattern races arise within Amazonia before spreading out and displacing ancestral subspecies. Further evidence from other species is now required to assess whether this pattern is a general feature of *Heliconius*, and indeed for other, unrelated taxa. Intriguingly, a number of *Heliconius* species also exhibit very similar colour patterns but are for the most part allopatric (e.g. *H. clysonymus*, *H. hierax*, *H. himera*, *H. hortense*, *H.ricini).* It seems possible that these species represent the remnants of an ancient and previously widespread mimicry ring that has now been surpassed by new colour patterns and mimicry rings. Finally, understanding the initial evolution of new colour patterns that arise in the face of stabilising selection remains an unresolved problem and devising convincing ways to test competing explanations must surely represent a major goal for those concerned with the biogeography and speciation of heliconiines.

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Appendix 1

Range maps for the heliconiines are presented from page 151-177. A revised version of the taxonomy presented by Lamas (2004) was applied; table A.1.1 shows were the two differ. Unless a reference is given which provides evidence in support of the adopted taxonomy, I treated names as synonyms where I found wing pattern characters to be too variable to permit putative taxa to be consistently distinguished. However, since this is not intended to be a taxonomic revision, I do not make formal taxonomic changes here. Table A.2.2 shows the altitudinal bounds used to clip species ranges. Maps represent species' native ranges, with two exceptions; *Dryadula phaetusa* and *Agraulis vanillae incarnata* have been introduced to southern Florida and the Hawaiian archipelago, respectively (Waage et al. 1981). Consequently, their presence in these regions was ignored when conducting the analyses presented in chapters 2 and 3.

Table A.1.1. Nomenclature where different from Lamas (2004).

Adopted taxonomy	Former taxonomy or names treated as synonyms	Reference
Eueides heliconioides eanes W.C. Hewitson 1861	*Eueides heliconioides koenigi* H. Holzinger & R. Holzinger 1993	
Eueides lampeto acacetes W.C. Hewitson 1869	*Eueides lampeto concisa* G. Lamas 1985	
Eueides tales subsp. nov. Brazil, Rondônia	-	Brown (1979)
Eueides tales subsp. nov. Colombia	-	LeCrom, pers. comm.
Heliconius burneyi huebneri O. Staudinger 1897	*Heliconius burneyi ada* H. Neustetter 1925	
Heliconius burneyi huebneri O. Staudinger 1897	*Heliconius burneyi anjae* W.M. Neukirchen 1995	
Heliconius burneyi huebneri O. Staudinger 1897	*Heliconius burneyi boliviensis* W.M. Neukirchen 1995	
Heliconius burneyi huebneri O. Staudinger 1897	*Heliconius burneyi koenigi* W.M. Neukirchen 1995	

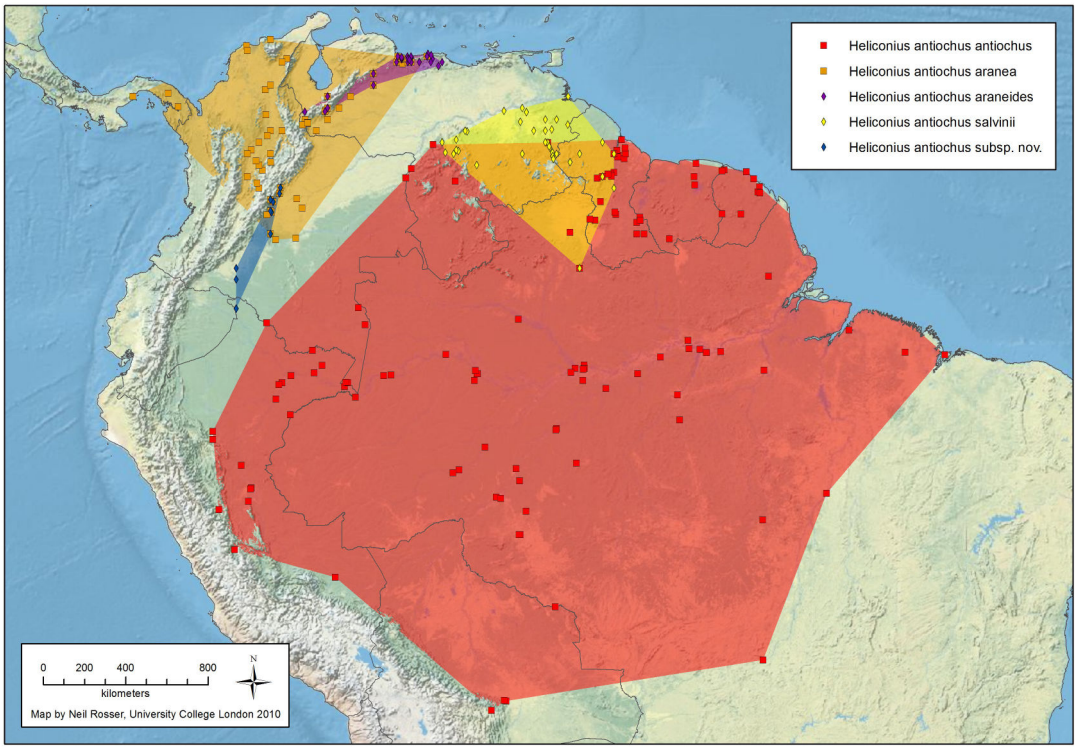
Heliconius burneyi mirtarosa A.M. Orellana 2006	-	Orellana (2006)
Heliconius chestertonii (W.C. Hewitson, 1872)	*Heliconius erato chestertonii* (W.C. Hewitson, 1872)	Arias *et al.* (2008)
Heliconius demeter demeter O. Staudinger 1897	*Heliconius demeter angeli* W.M. Neukirchen 1997	
Heliconius demeter demeter O. Staudinger 1897	*Heliconius demeter subsp. nov.* Peru	
Heliconius egeria egerides O. Staudinger 1897	*Heliconius egeria christiani* W.M. Neukirchen 1997	
Heliconius egeria hyas G. Weymer 1883	*Heliconius egeria mariasibyllae* W.M. Neukirchen 1991	
Heliconius elevatus elevatus E. Nöldner 1901	*Heliconius elevatus willmotti* W.M. Neukirchen 1997	
Heliconius elevatus tumatumari W.J. Kaye 1906	*Heliconius elevatus sonjae* W.M. Neukirchen 1997	
Heliconius eratosignis eratosignis (J.J. Joicey & G. Talbot 1925)	*Heliconius demeter eratosignis* (J.J. Joicey & G. Talbot 1925)	Mallet et al., unpub. data
Heliconius eratosignis tambopata G. Lamas 1985	*Heliconius demeter tambopata* G. Lamas 1985	Mallet et al., unpub. data
Heliconius eratosignis ucayalensis H. Holzinger & R. Holzinger 1975	*Heliconius demeter ucayalensis* H. Holzinger & R. Holzinger 1975	Mallet et al., unpub. data
Heliconius eratosignis ulysses K.S. Brown & W.W. Benson 1975	*Heliconius demeter ulysses* K.S. Brown & W.W. Benson 1975	Mallet et al., unpub. data
Heliconius hecale felix G. Weymer 1894	*Heliconius hecale zeus* W.M. Neukirchen 1995	
Heliconius hecale vetustus A.G. Butler 1873	*Heliconius hecale naxos* W.M. Neukirchen 1998	
Heliconius hecuba choarina (W.C. Hewitson 1872)	*Heliconius hecuba bonplandi* W.M. Neukirchen 1991	
Heliconius hecuba choarina (W.C. Hewitson 1872)	*Heliconius hecuba lamasi* W.M. Neukirchen 1991	
Heliconius hecuba crispus O. Staudinger 1885	*Heliconius hecuba salazari* W.M. Neukirchen 1993	
Heliconius hecuba crispus O. Staudinger 1885	*Heliconius hecuba walteri* J.A. Salazar 1998	
Heliconius leucadia pseudorhea O. Staudinger 1897	*Heliconius leucadia andromeda* W.M. Neukirchen 1996	
Heliconius leucadia pseudorhea O. Staudinger 1897	*Heliconius leucadia birgitae* W.M. Neukirchen 1996	
Heliconius pachinus O. Salvin 1871	*Heliconius cydno pachinus* O. Salvin 1871	
Heliconius hewitsoni (W.C. Hewitson 1875)	*Heliconius sapho hewitsoni* (W.C. Hewitson 1875)	

Heliconius telesiphe subsp. nov. Ecuador	-	Brown (1979)
Heliconius timareta florencia (N. Giraldo et al.)	-	Giraldo *et al.* (2008)
Heliconius timareta subsp. nov. Colombia	-	Pardo-Diaz *et al.* (in prep.)
Heliconius timareta subsp. nov. Ecuador	-	Brown (1979)
Heliconius timareta subsp. nov. Peru	-	Mallet (2009)
Neruda aoede bartletti (H. Druce 1876)	*Neruda aoede auca* W.M. Neukirchen 1997	
Neruda metis G.R.P. Moreira & C.G.C. Mielke 2010	-	Moreira & Mielke (2010)

Table A.1.2. Altitudinal bounds used to clip species ranges (in metres). For sources used see methods. * indicates that species range was not clipped due to lack of information on elevational range.

Taxon	lower bound	upper bound
Agraulis n. sp.	0	3100
Agraulis vanillae	0	3100
Dione glycera	1000	3650
Dione juno	0	3000
Dione moneta butleri / Dione moneta poeyii	500	3500
Dione moneta moneta	0	3500
Dryadula phaetusa	0	1550
Dryas iulia	0	2000
Eueides aliphera	0	1800
Eueides emsleyi	0	1200
Eueides heliconioides	0	1800
Eueides isabella	0	1500
Eueides lampeto	0	1700
Eueides libitina	*	*
Eueides lineata	0	1850
Eueides lybia	0	1200
Eueides pavana	0	1600
Eueides procula	0	2000
Eueides tales	0	1500
Eueides vibilia	0	1500
Heliconius antiochus	0	1200
Heliconius astraea	0	1200
Heliconius atthis	0	1900
Heliconius besckei	0	2500
Heliconius burneyi	0	1000
Heliconius charithonia	0	2000
Heliconius chestertonii	0	2200
Heliconius clysonymus	800	2500

Heliconius congener	500	2100
Heliconius cydno	0	2100
Heliconius demeter	0	1100
Heliconius egeria	0	1200
Heliconius eleuchia	0	2000
Heliconius elevatus	0	2000
Heliconius erato	0	2200
Heliconius eratosignis	0	1100
Heliconius ethilla	0	2000
Heliconius hecale	0	1900
Heliconius hecalesia	0	1800
Heliconius hecuba	900	2400
Heliconius hermathena	*	*
Heliconius heurippa	800	1800
Heliconius hewitsoni	0	1400
Heliconius hierax	400	2000
Heliconius himera	400	2500
Heliconius hortense	0	2200
Heliconius ismenius	0	1500
Heliconius lalitae	*	*
Heliconius leucadia	0	1000
Heliconius luciana	0	1600
Heliconius melpomene	0	1800
Heliconius nattereri	0	1300
Heliconius numata	0	1800
Heliconius pachinus	0	1600
Heliconius pardalinus	0	1200
Heliconius peruvianus	0	2000
Heliconius ricini	*	*
Heliconius sapho	0	1700
Heliconius sara	0	1300
Heliconius telesiphe	600	2500
Heliconius timareta	800	1800
Heliconius tristero	*	*
Heliconius wallacei	0	1200
Heliconius xanthocles	0	1500
Laparus doris	0	1500
Neruda aoede	0	1350
Neruda godmani	0	1200
Neruda metharme	0	1300
Neruda metis	*	*
Podotricha judith	1000	2600
Podotricha telesiphe	800	2500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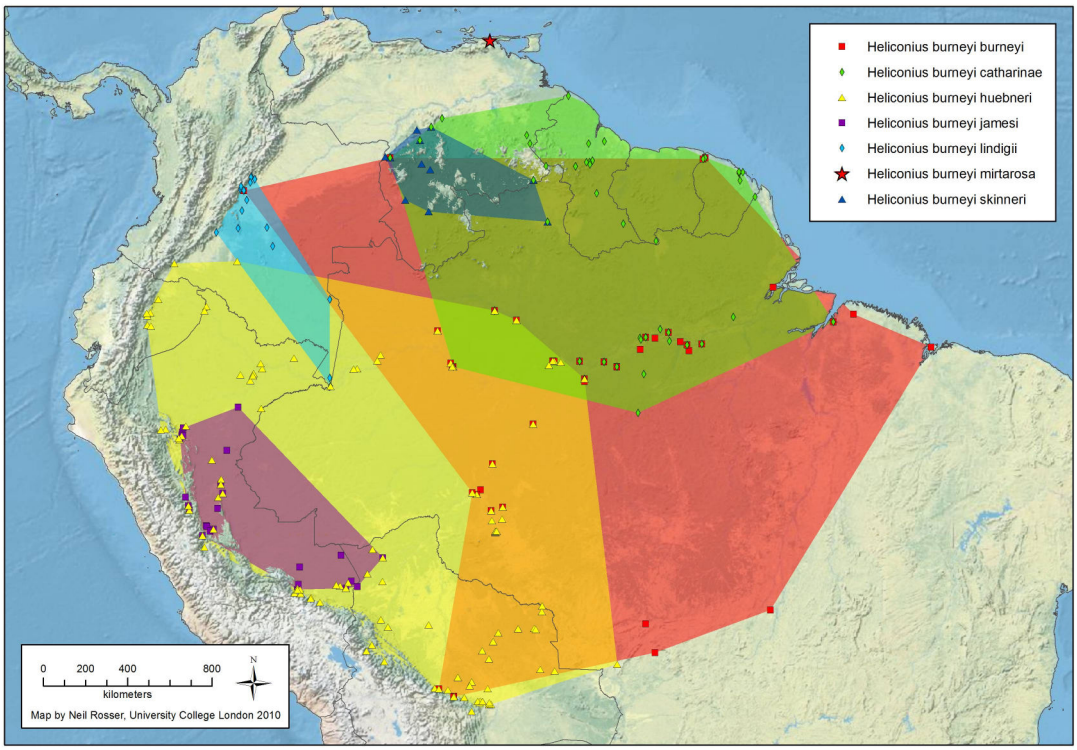
Heliconius antiochus aranea
Heliconius antiochus araneides
Heliconius antiochus salvinii
Heliconius antiochus subsp. nov.
0 200 400 800
kilometers
N
Map by Neil Rosser, University College London 2010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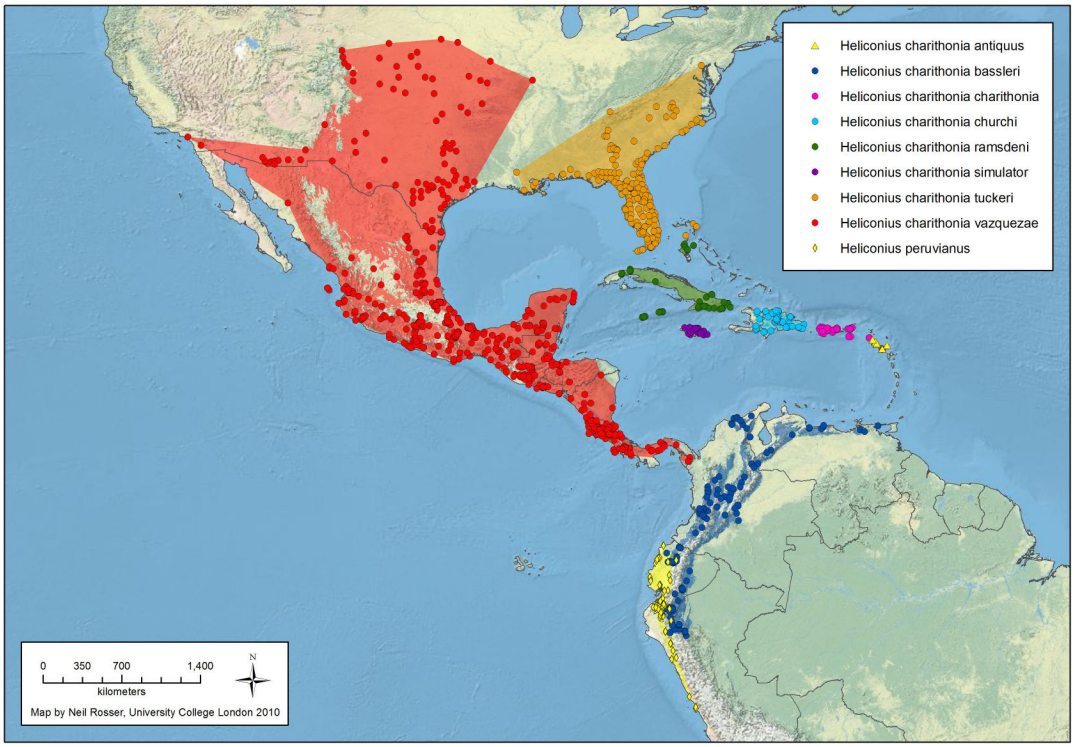
Heliconius burneyi catharinae
Heliconius burneyi huebneri
Heliconius burneyi jamesi
Heliconius burneyi lindigii
Heliconius burneyi mirtarosa
Heliconius burneyi skinneri
0 200 400 800
kilometers
N
Map by Neil Rosser, University College London 2010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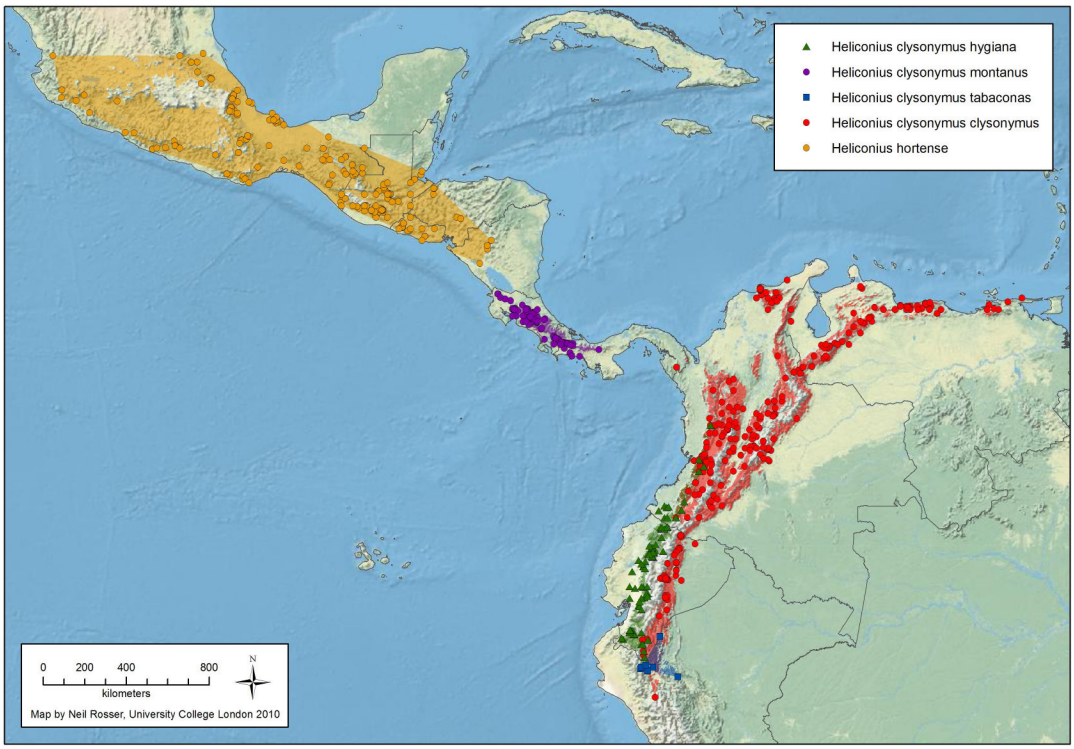
Heliconius charithonia bassleri
Heliconius charithonia charithonia
Heliconius charithonia churchi
Heliconius charithonia ramsdeni
Heliconius charithonia simulator
Heliconius charithonia tuckeri
Heliconius charithonia vazquezae
Heliconius peruvianus
0 350 700 1,400
kilometers
N
Map by Neil Rosser, University College London 2010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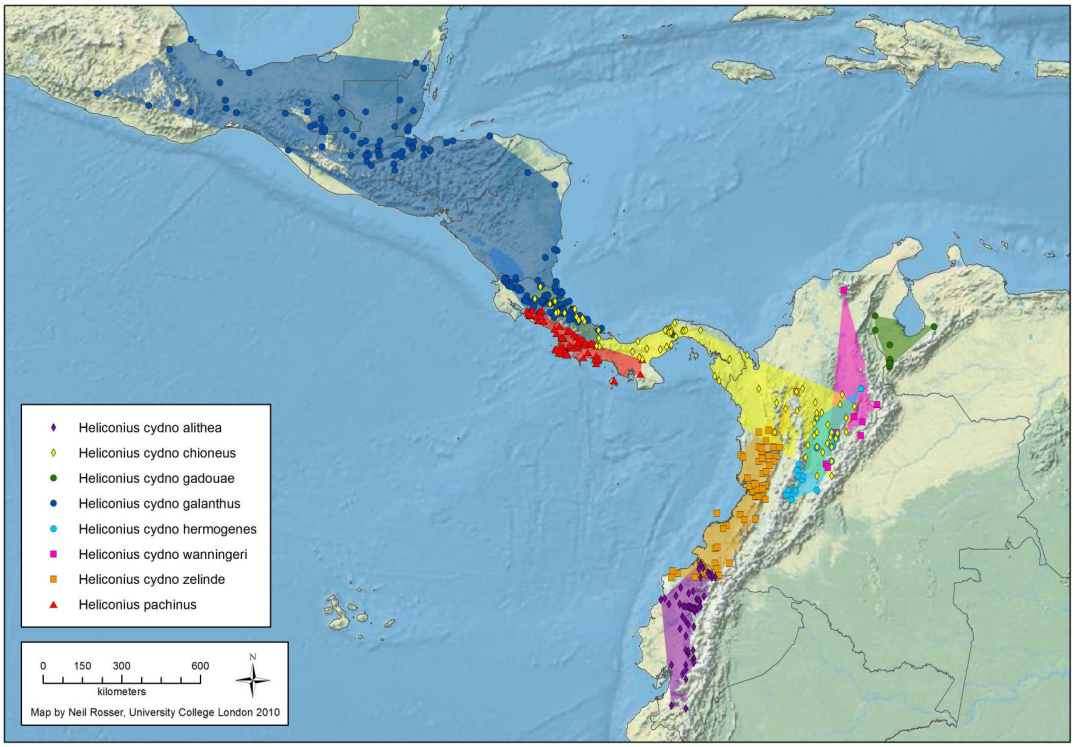
Heliconius clysonymus montanus
Heliconius clysonymus tabaconas
Heliconius clysonymus clysonymus
Heliconius hortense
0 200 400 800
kilometers
N
Map by Neil Rosser, University College London 2010


Heliconius cydno alithea
Heliconius cydno chioneus
Heliconius cydno gadouae
Heliconius cydno galanthus
Heliconius cydno hermogenes
Heliconius cydno wanningeri
Heliconius cydno zelinde
Heliconius pachinus
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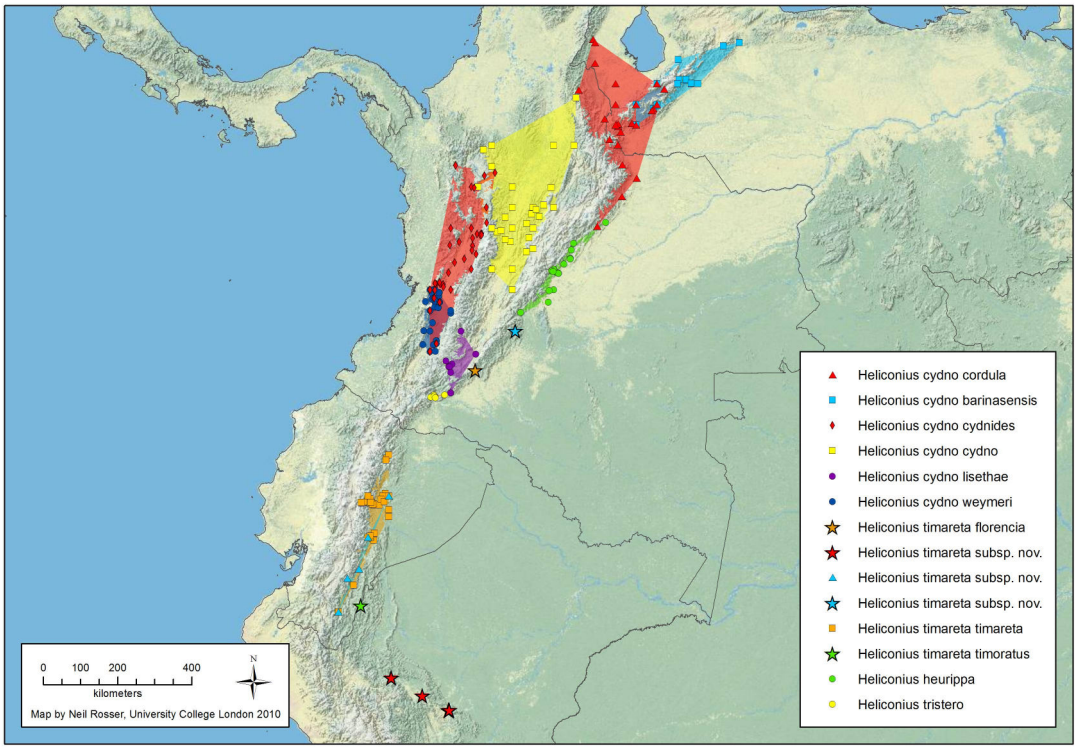
kilometers
N
Map by Neil Rosser, University College London 2010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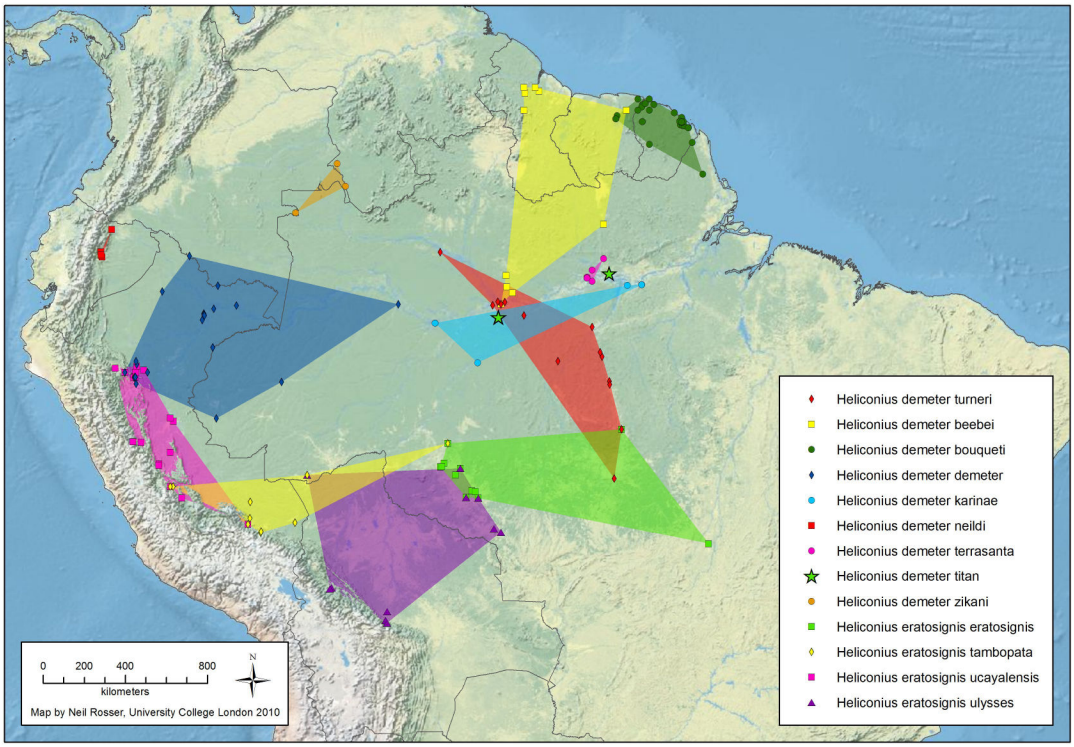
Heliconius cydno barinasensis
Heliconius cydno cydnides
Heliconius cydno cydno
Heliconius cydno lisethae
Heliconius cydno weymeri
Heliconius timareta florencia
Heliconius timareta subsp. nov.
Heliconius timareta subsp. nov.
Heliconius timareta subsp. nov.
Heliconius timareta timareta
Heliconius timareta timoratus
Heliconius heurippa
Heliconius tristero
0 100 200 400
kilometers
N
Map by Neil Rosser, University College London 2010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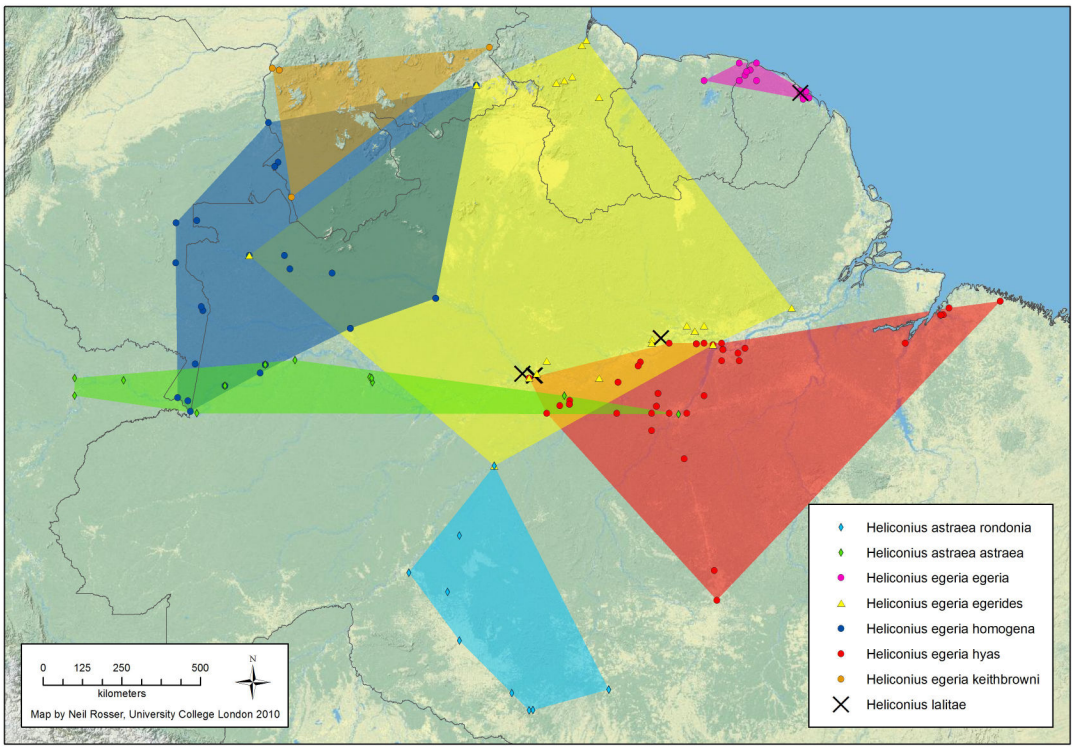
Heliconius demeter beebei
Heliconius demeter bouqueti
Heliconius demeter demeter
Heliconius demeter karinae
Heliconius demeter neildi
Heliconius demeter terrasanta
Heliconius demeter titan
Heliconius demeter zikani
Heliconius eratosignis eratosignis
Heliconius eratosignis tambopata
Heliconius eratosignis ucayalensis
Heliconius eratosignis ulysses
0 200 400 800
kilometers
N
Map by Neil Rosser, University College London 2010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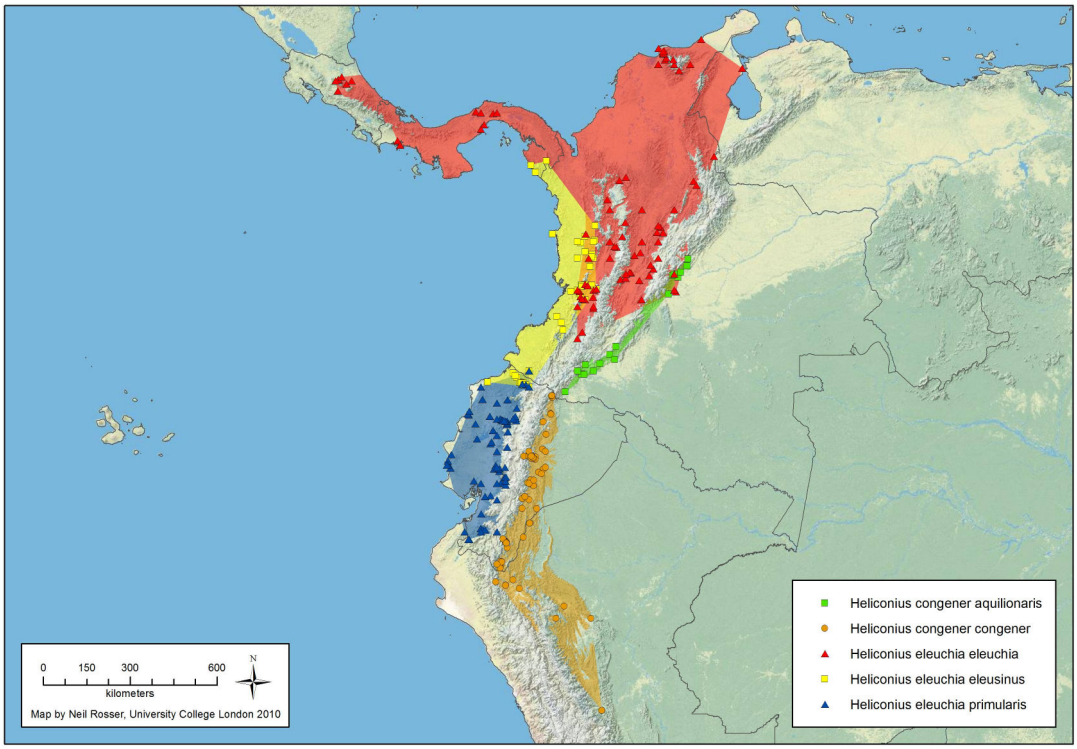
Heliconius astraea astraea
Heliconius egeria egeria
Heliconius egeria egerides
Heliconius egeria homogena
Heliconius egeria hyas
Heliconius egeria keithbrowni
Heliconius lalitae
0 125 250 500
kilometers
N
Map by Neil Rosser, University College London 2010


Heliconius congener aquilionaris
Heliconius congener congener
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Heliconius eleuchia eleusinus
Heliconius eleuchia primularis
0 150 300 600
kilometers
N
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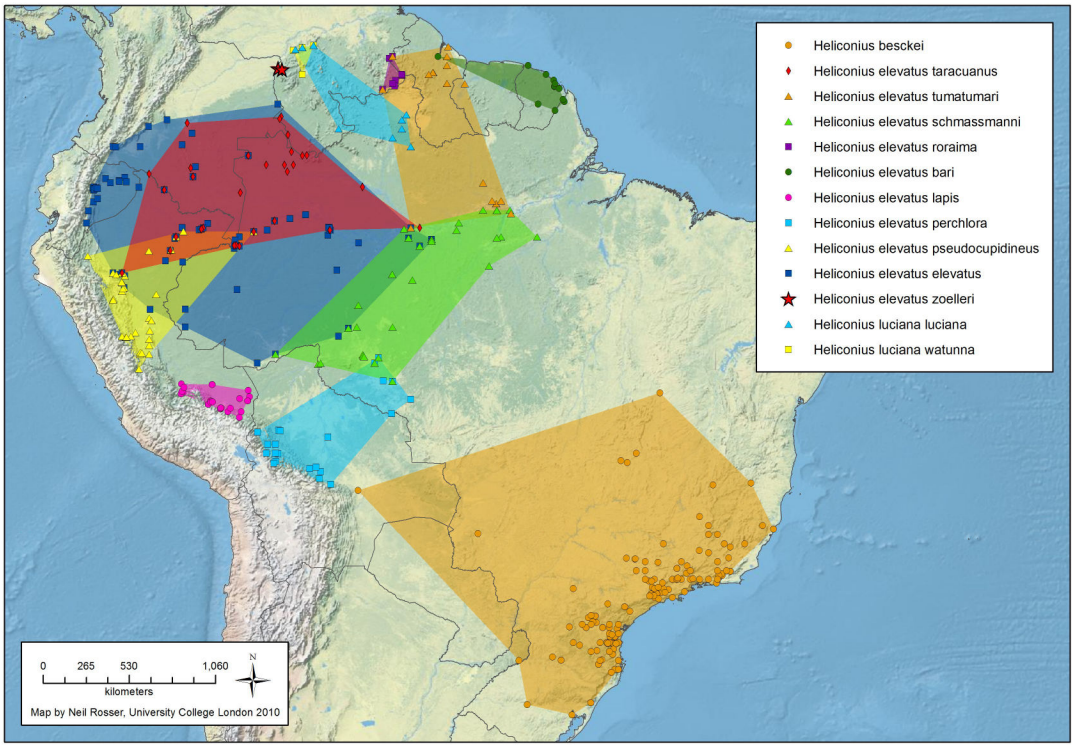
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Heliconius elevatus taracuanus
Heliconius elevatus tumatumari
Heliconius elevatus schmassmanni
Heliconius elevatus roraima
Heliconius elevatus bari
Heliconius elevatus lapis
Heliconius elevatus perchlora
Heliconius elevatus pseudocupidineus
Heliconius elevatus elevatus
Heliconius elevatus zoelleri
Heliconius luciana luciana
Heliconius luciana watunna
0 265 530 1,060
kilometers
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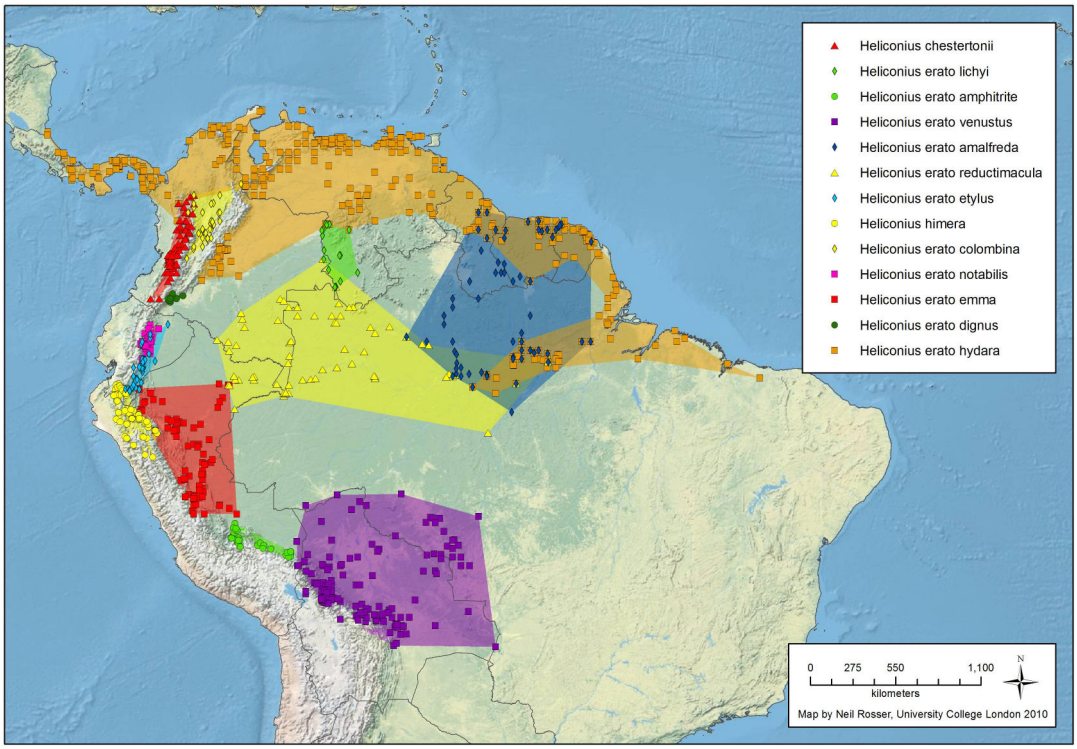
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Heliconius erato amphitrite
Heliconius erato venustus
Heliconius erato amalfreda
Heliconius erato reductimacula
Heliconius erato etylus
Heliconius himera
Heliconius erato colombina
Heliconius erato notabilis
Heliconius erato emma
Heliconius erato dignus
Heliconius erato hydara
0 275 550 1,100
kilometers
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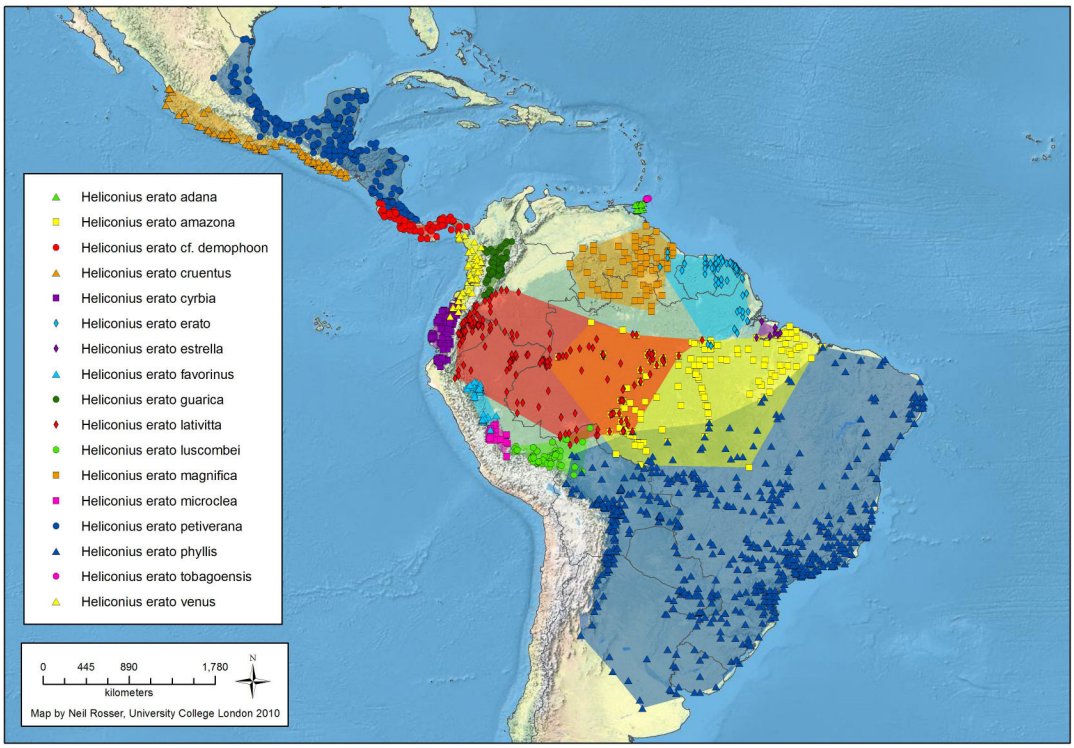
Heliconius erato adana
Heliconius erato amazona
Heliconius erato cf. demophoon
Heliconius erato cruentus
Heliconius erato cyrbia
Heliconius erato erato
Heliconius erato estrella
Heliconius erato favorinus
Heliconius erato guarica
Heliconius erato lativitta
Heliconius erato luscombei
Heliconius erato magnifica
Heliconius erato microclea
Heliconius erato petiverana
Heliconius erato phyllis
Heliconius erato tobagoensis
Heliconius erato venus
0 445 890 1,780
kilometers
N
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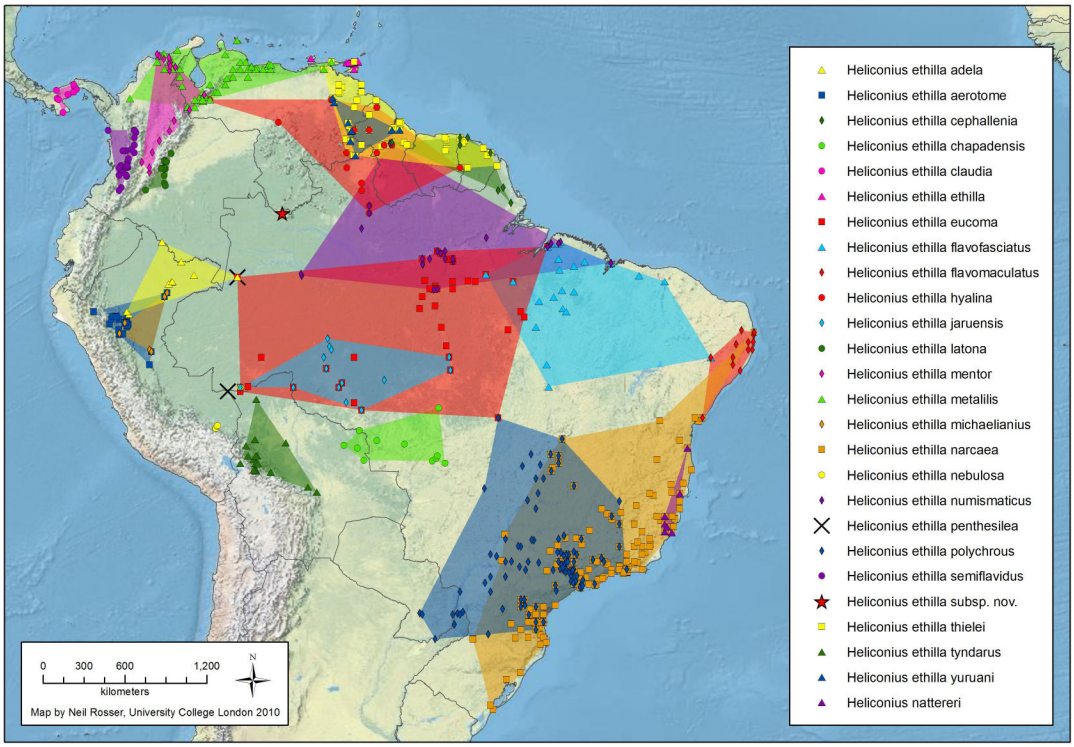
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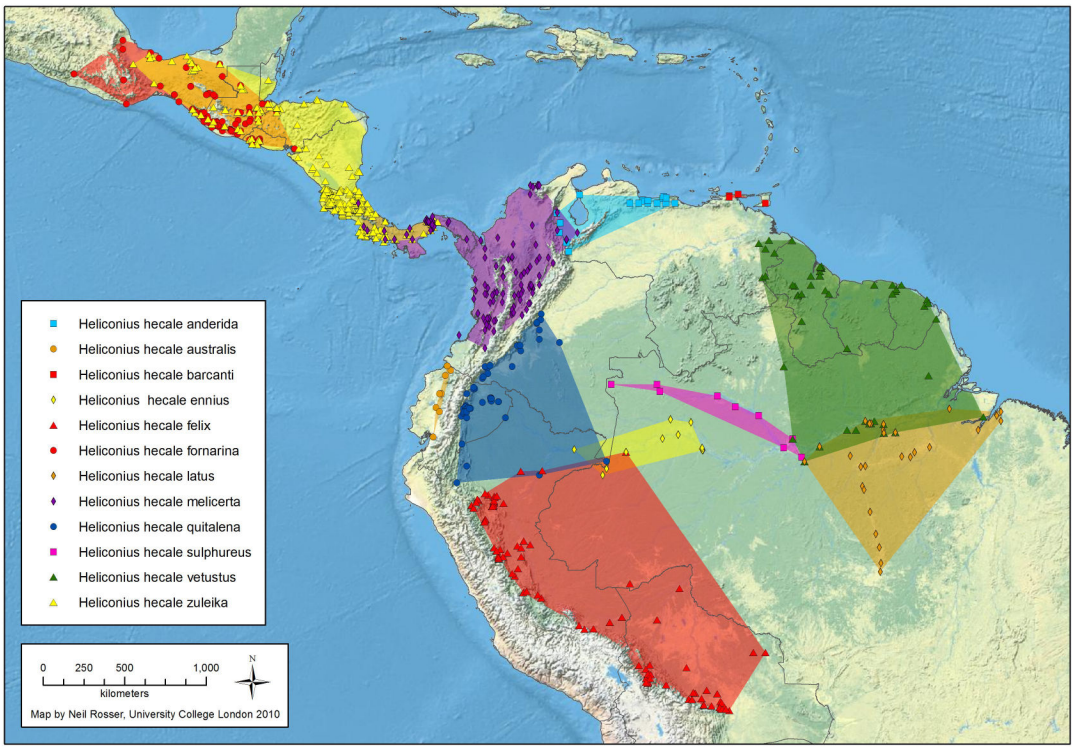
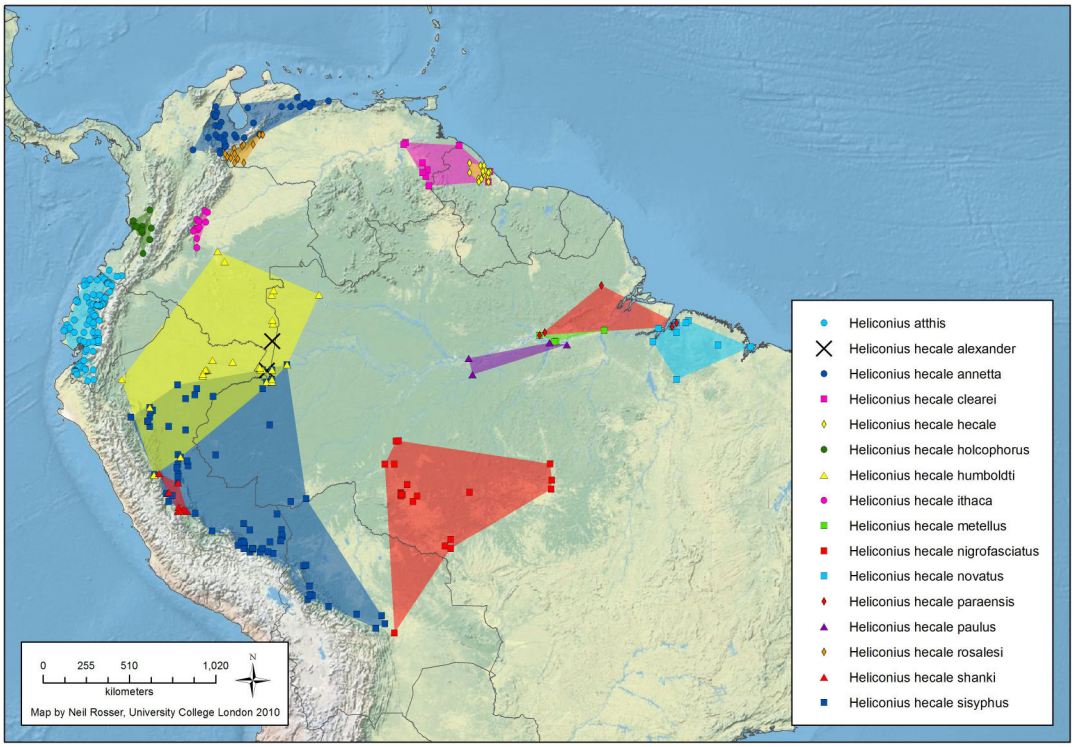
Heliconius ethilla aerotome
Heliconius ethilla cephallenia
Heliconius ethilla chapadensis
Heliconius ethilla claudia
Heliconius ethilla ethilla
Heliconius ethilla eucoma
Heliconius ethilla flavofasciatus
Heliconius ethilla flavomaculatus
Heliconius ethilla hyalina
Heliconius ethilla jaruensis
Heliconius ethilla latona
Heliconius ethilla mentor
Heliconius ethilla metalilis
Heliconius ethilla michaelianius
Heliconius ethilla narcaea
Heliconius ethilla nebulosa
Heliconius ethilla numismaticus
Heliconius ethilla penthesilea
Heliconius ethilla polychrous
Heliconius ethilla semiflavidus
Heliconius ethilla subsp. nov.
Heliconius ethilla thielei
Heliconius ethilla tyndarus
Heliconius ethilla yuruani
Heliconius nattereri
0 300 600 1,200
kilometers
N
Map by Neil Rosser, University College London 2010


Heliconius hecale anderida
Heliconius hecale australis
Heliconius hecale barcanti
Heliconius hecale ennius
Heliconius hecale felix
Heliconius hecale fornarina
Heliconius hecale latus
Heliconius hecale melicerta
Heliconius hecale quitalena
Heliconius hecale sulphureus
Heliconius hecale vetustus
Heliconius hecale zuleika
0 250 500 1,000
kilometers
N
Map by Neil Rosser, University College London 2010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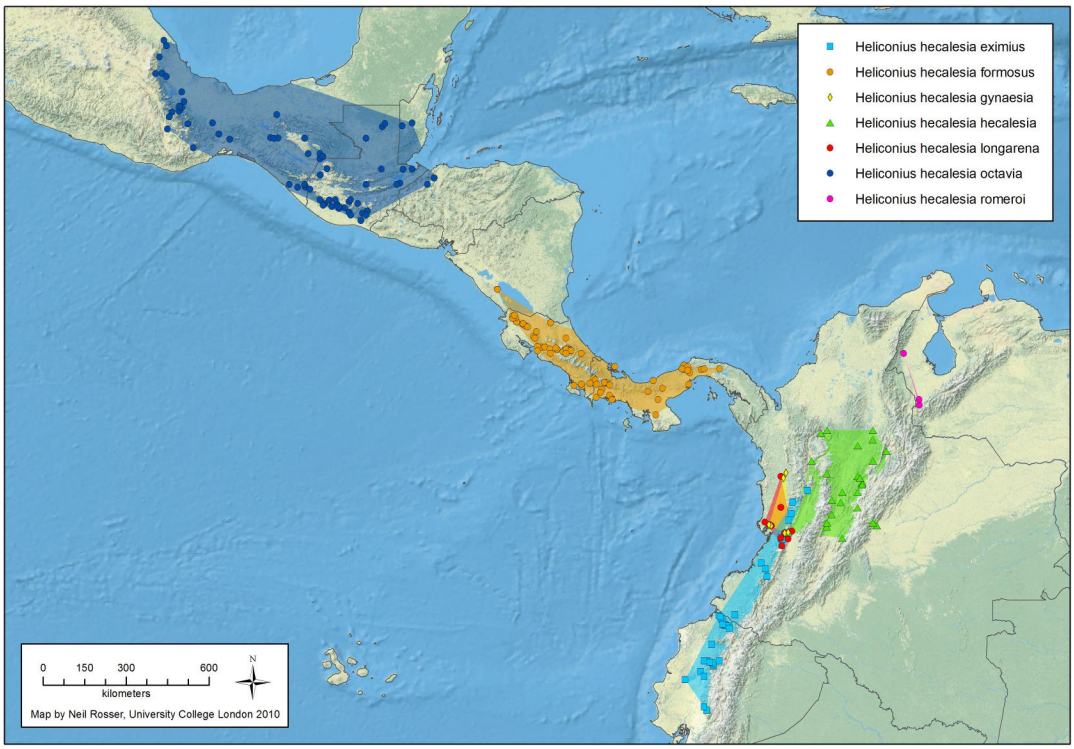
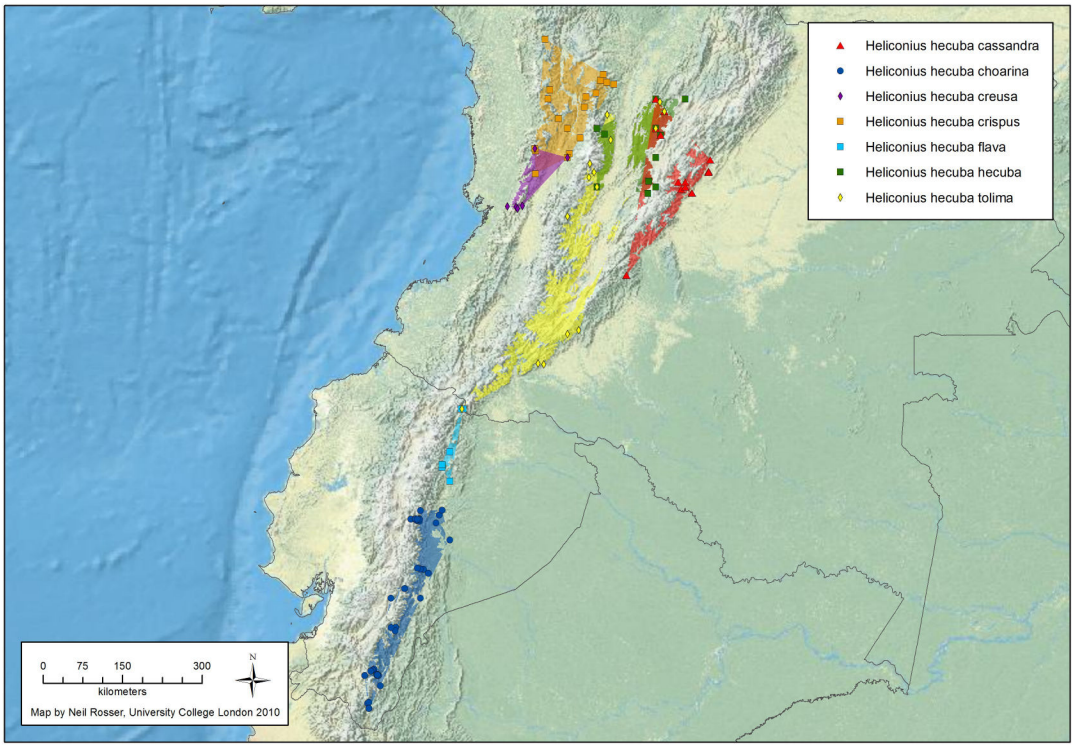
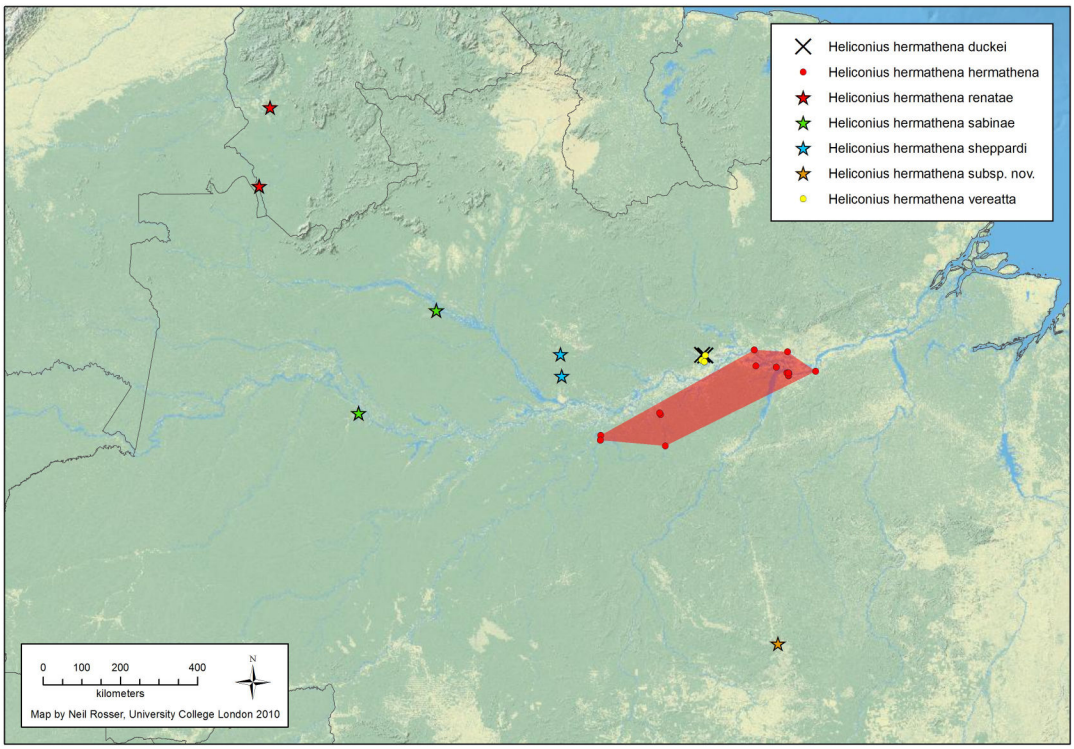
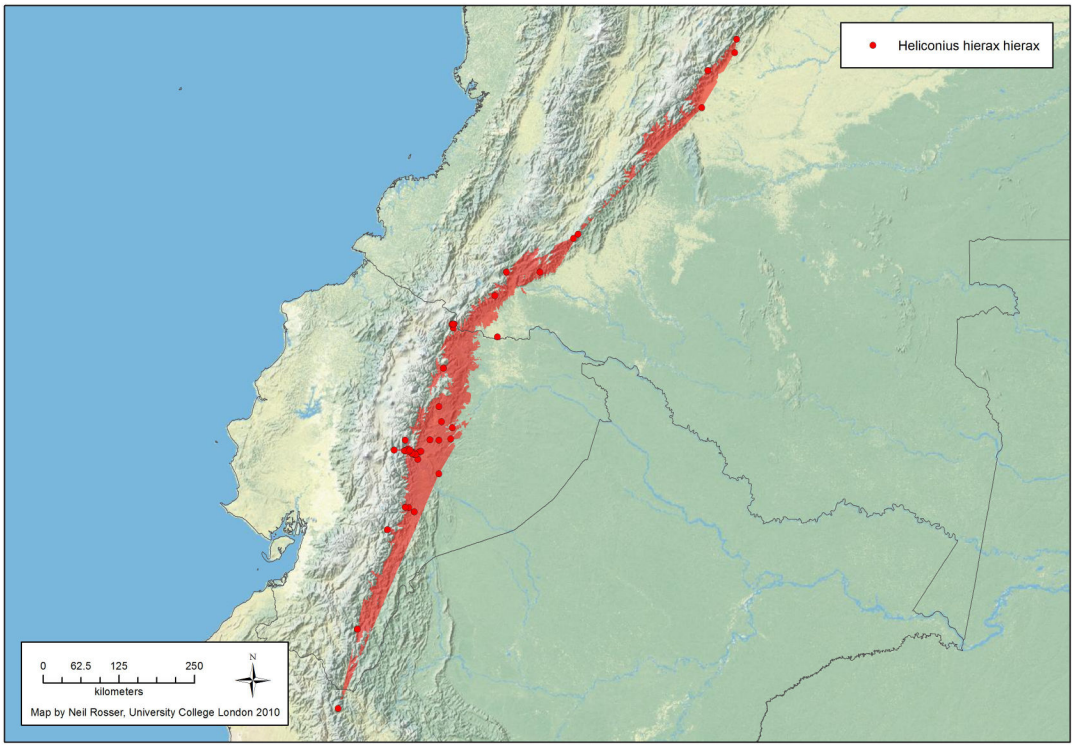
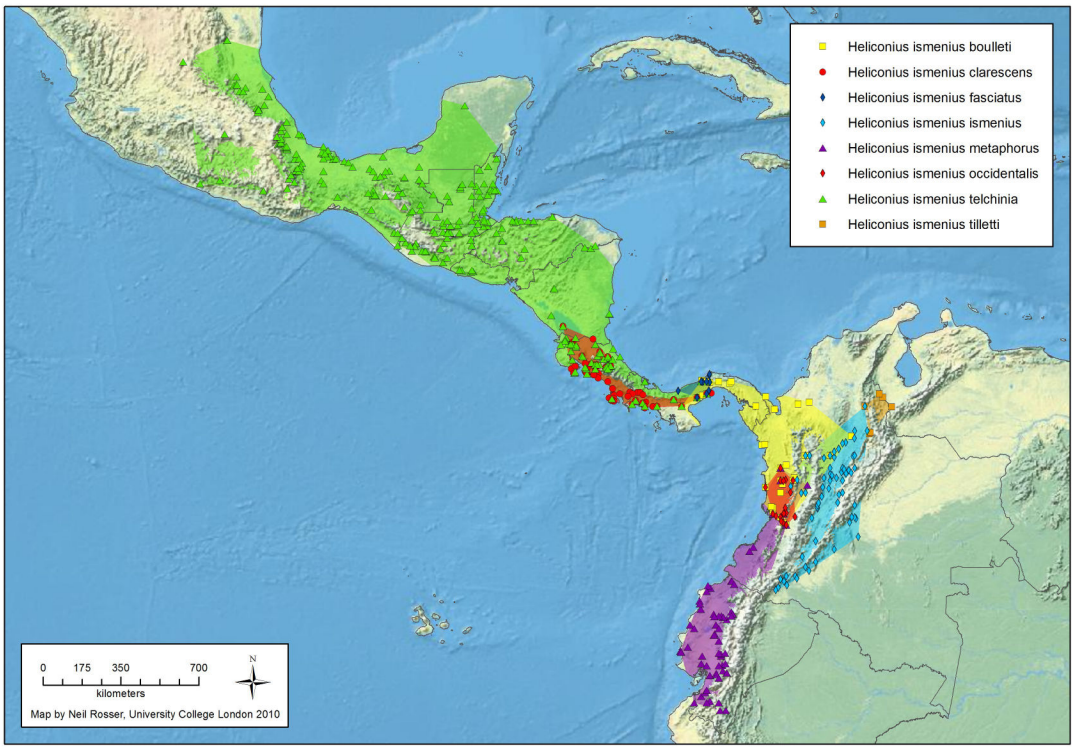
Heliconius hecale alexander
Heliconius hecale annetta
Heliconius hecale clearei
Heliconius hecale hecale
Heliconius hecale holcophorus
Heliconius hecale humboldti
Heliconius hecale ithaca
Heliconius hecale metellus
Heliconius hecale nigrofasciatus
Heliconius hecale novatus
Heliconius hecale paraensis
Heliconius hecale paulus
Heliconius hecale rosalesi
Heliconius hecale shanki
Heliconius hecale sisyphus
0 255 510 1,020
kilometers
N
Map by Neil Rosser, University College London 2010

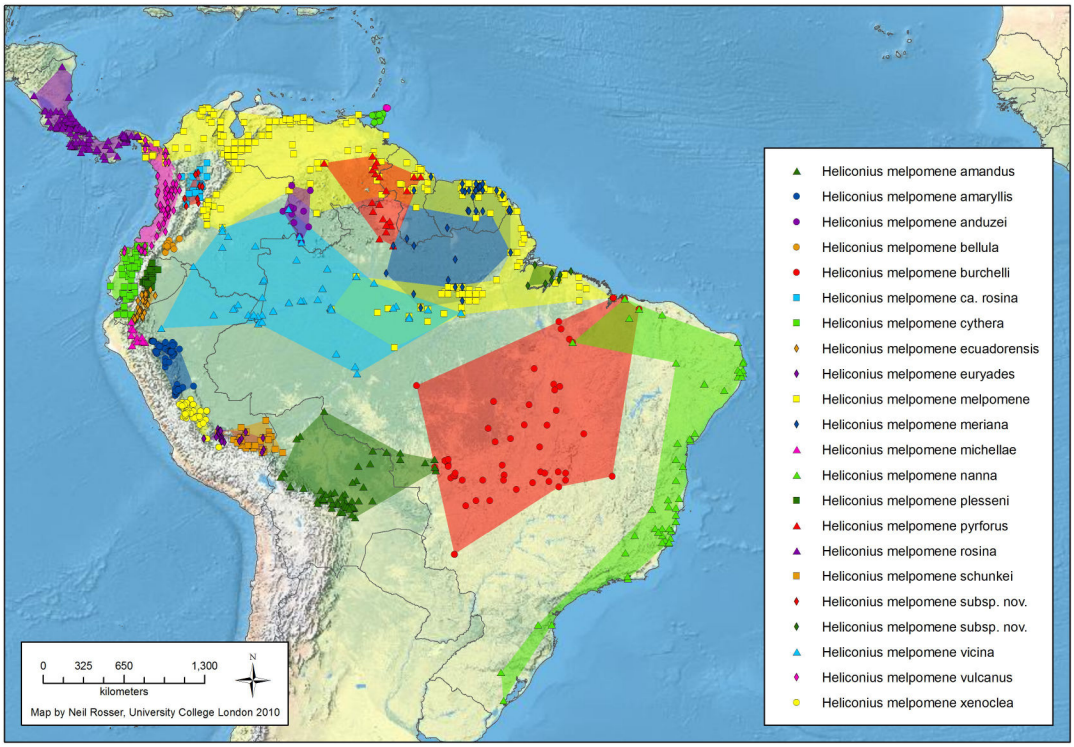

Heliconius hecalesia eximius
Heliconius hecalesia formosus
Heliconius hecalesia gynaesia
Heliconius hecalesia hecalesia
Heliconius hecalesia longarena
Heliconius hecalesia octavia
Heliconius hecalesia romeroi
0 150 300 600
kilometers
N
Map by Neil Rosser, University College London 2010

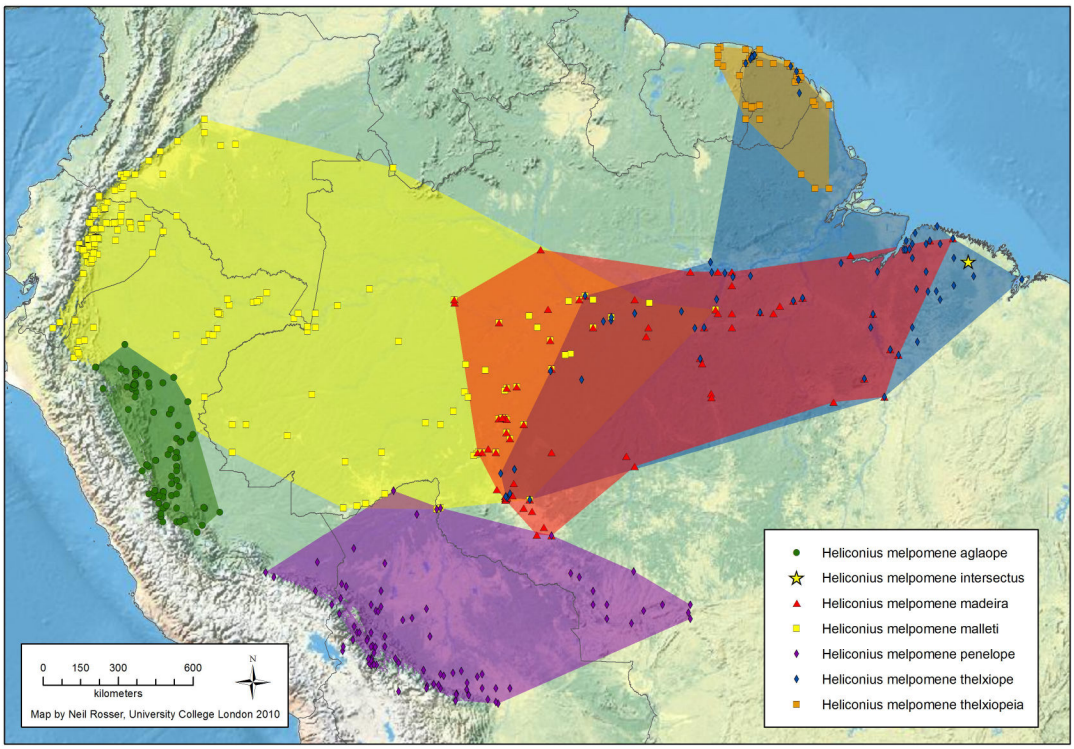

Heliconius hecuba cassandra
Heliconius hecuba choarina
Heliconius hecuba creusa
Heliconius hecuba crispus
Heliconius hecuba flava
Heliconius hecuba hecuba
Heliconius hecuba tolima
0 75 150 300
kilometers
N
Map by Neil Rosser, University College London 2010

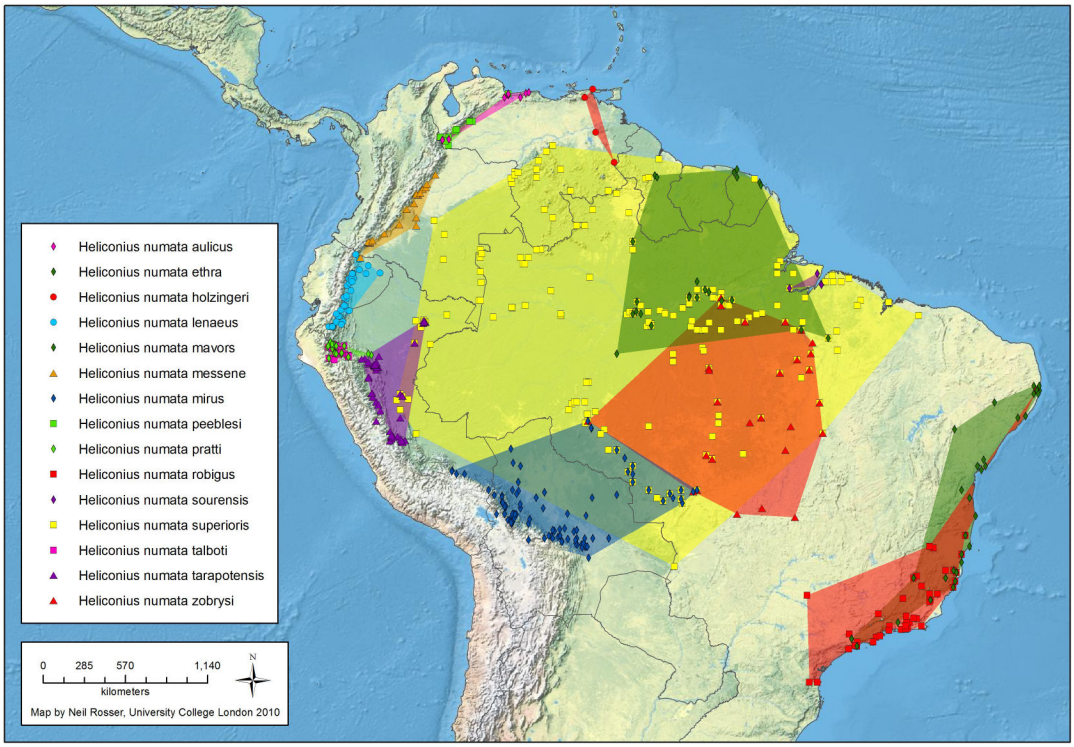

Heliconius hermathena duckei
Heliconius hermathena hermathena
Heliconius hermathena renatae
Heliconius hermathena sabinae
Heliconius hermathena sheppardi
Heliconius hermathena subsp. nov.
Heliconius hermathena vereatta
0 100 200 400
kilometers
N
Map by Neil Rosser, University College London 2010

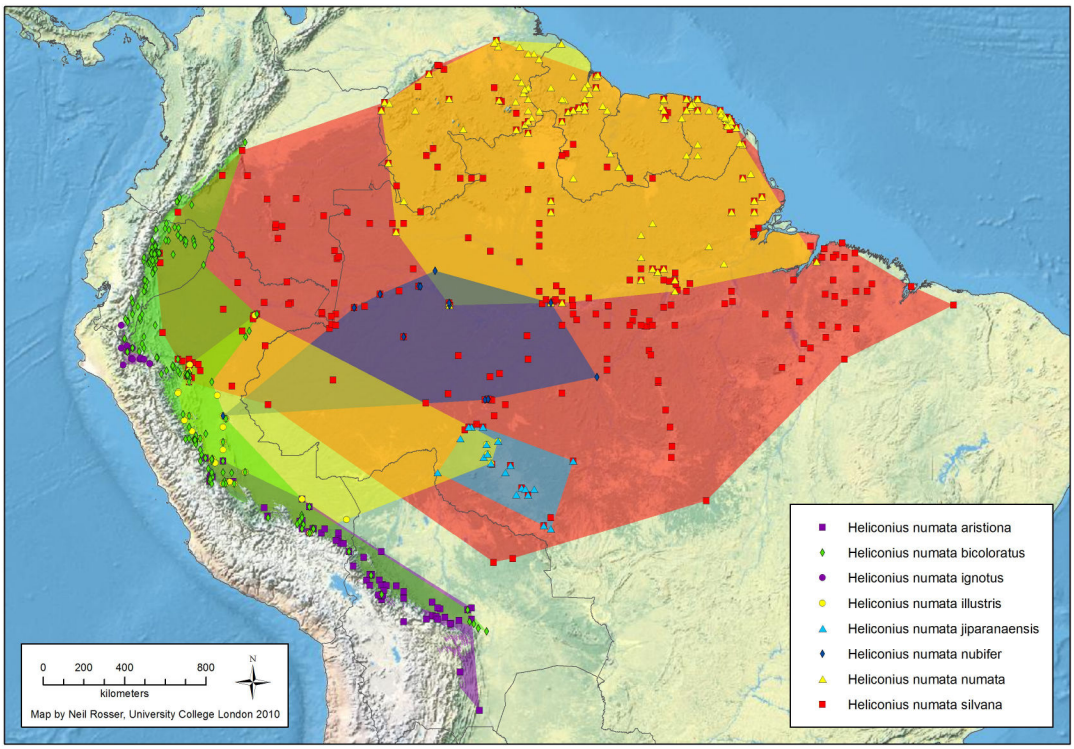

Heliconius hierax hierax
0 62.5 125 250
kilometers
N
Map by Neil Rosser, University College London 2010

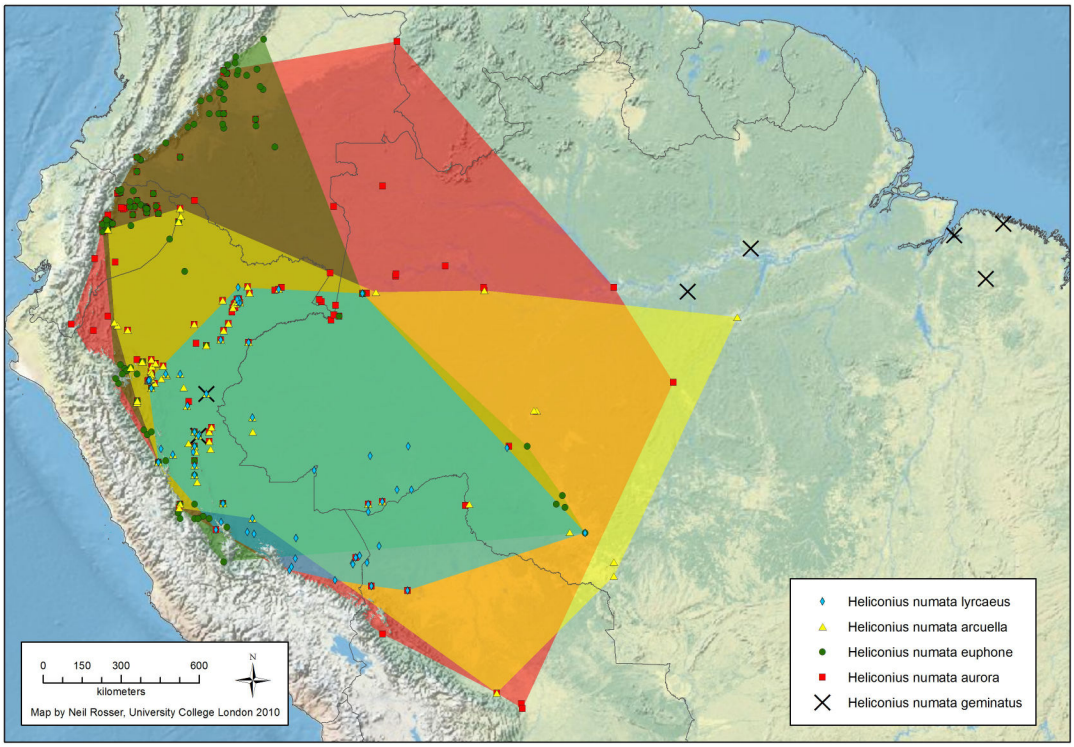

Heliconius ismenius boulleti
Heliconius ismenius clarescens
Heliconius ismenius fasciatus
Heliconius ismenius ismenius
Heliconius ismenius metaphorus
Heliconius ismenius occidentalis
Heliconius ismenius telchinia
Heliconius ismenius tilletti
0 175 350 700
kilometers
N
Map by Neil Rosser, University College London 2010

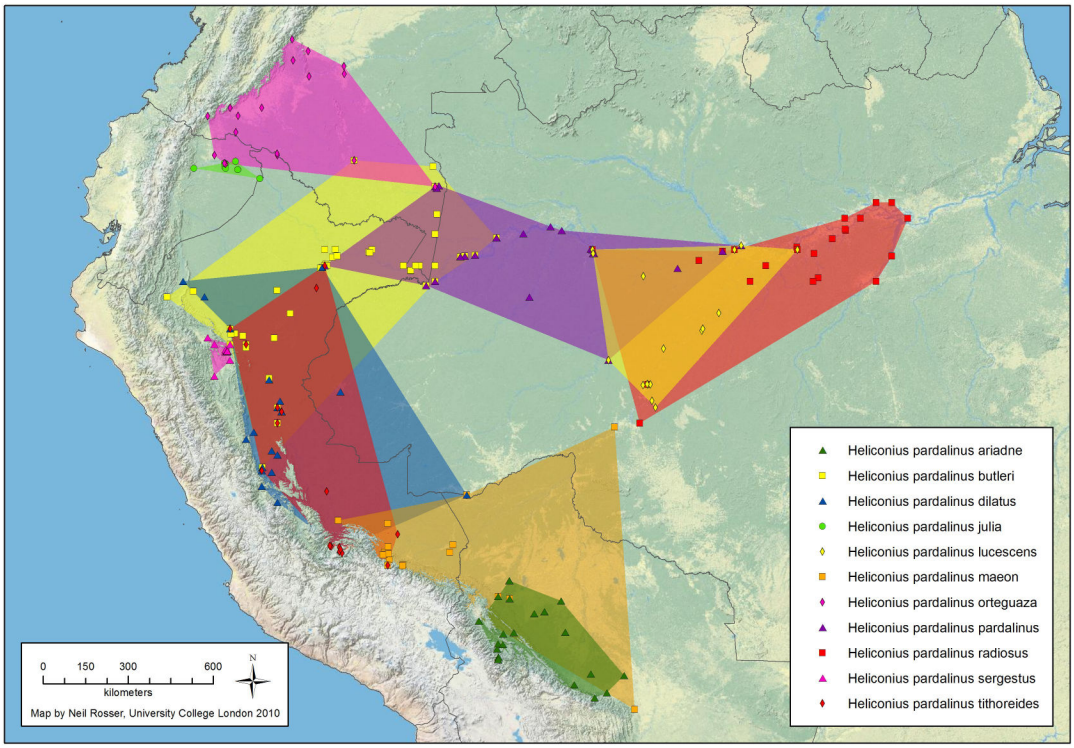

Heliconius melpomene amandus
Heliconius melpomene amaryllis
Heliconius melpomene anduzei
Heliconius melpomene bellula
Heliconius melpomene burchelli
Heliconius melpomene ca. rosina
Heliconius melpomene cythera
Heliconius melpomene ecuadorensis
Heliconius melpomene euryades
Heliconius melpomene melpomene
Heliconius melpomene meriana
Heliconius melpomene michellae
Heliconius melpomene nanna
Heliconius melpomene plesseni
Heliconius melpomene pyrforus
Heliconius melpomene rosina
Heliconius melpomene schunkei
Heliconius melpomene subsp. nov.
Heliconius melpomene subsp. nov.
Heliconius melpomene vicina
Heliconius melpomene vulcanus
Heliconius melpomene xenoclea
0 325 650 1,300
kilometers
N
Map by Neil Rosser, University College London 2010

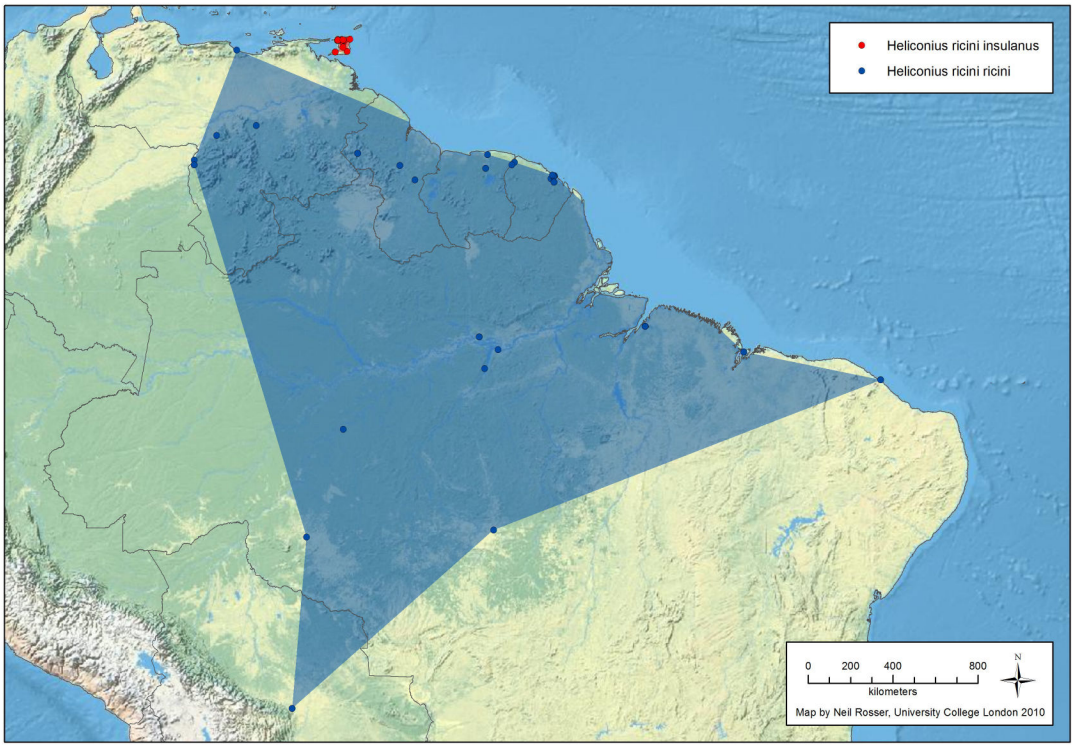

Heliconius melpomene aglaope
Heliconius melpomene intersectus
Heliconius melpomene madeira
Heliconius melpomene malleti
Heliconius melpomene penelope
Heliconius melpomene thelxiope
Heliconius melpomene thelxiopeia
0 150 300 600
kilometers
N
Map by Neil Rosser, University College London 2010

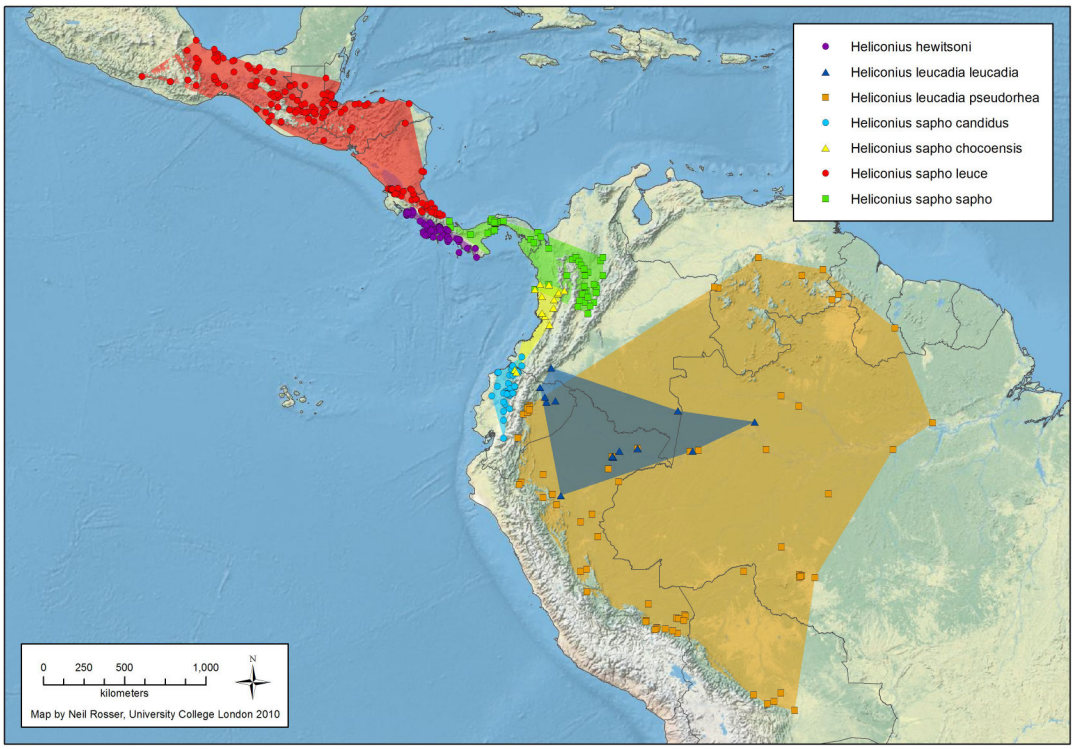

Heliconius numata aulicus
Heliconius numata ethra
Heliconius numata holzingeri
Heliconius numata lenaeus
Heliconius numata mavors
Heliconius numata messene
Heliconius numata mirus
Heliconius numata peeblesi
Heliconius numata pratti
Heliconius numata robigus
Heliconius numata sourensis
Heliconius numata superioris
Heliconius numata talboti
Heliconius numata tarapotensis
Heliconius numata zobrysi
0 285 570 1,140
kilometers
N
Map by Neil Rosser, University College London 2010

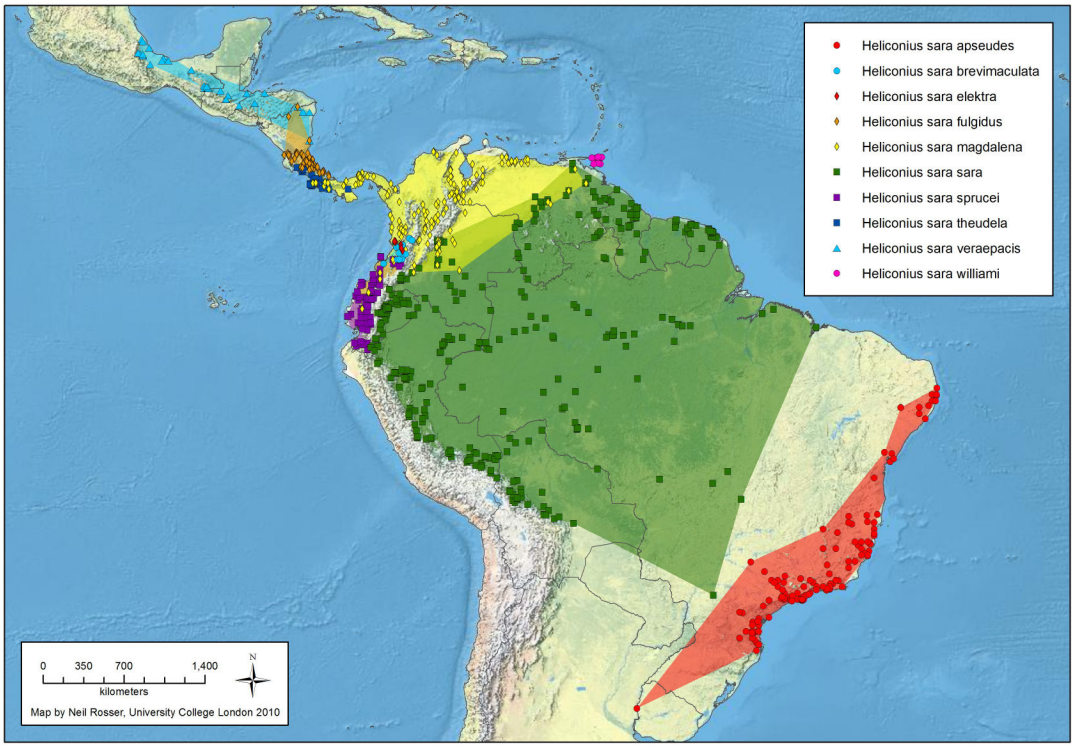

Heliconius numata aristiona
Heliconius numata bicoloratus
Heliconius numata ignotus
Heliconius numata illustris
Heliconius numata jiparanaensis
Heliconius numata nubifer
Heliconius numata numata
Heliconius numata silvana
0 200 400 800
kilometers
N
Map by Neil Rosser, University College London 2010

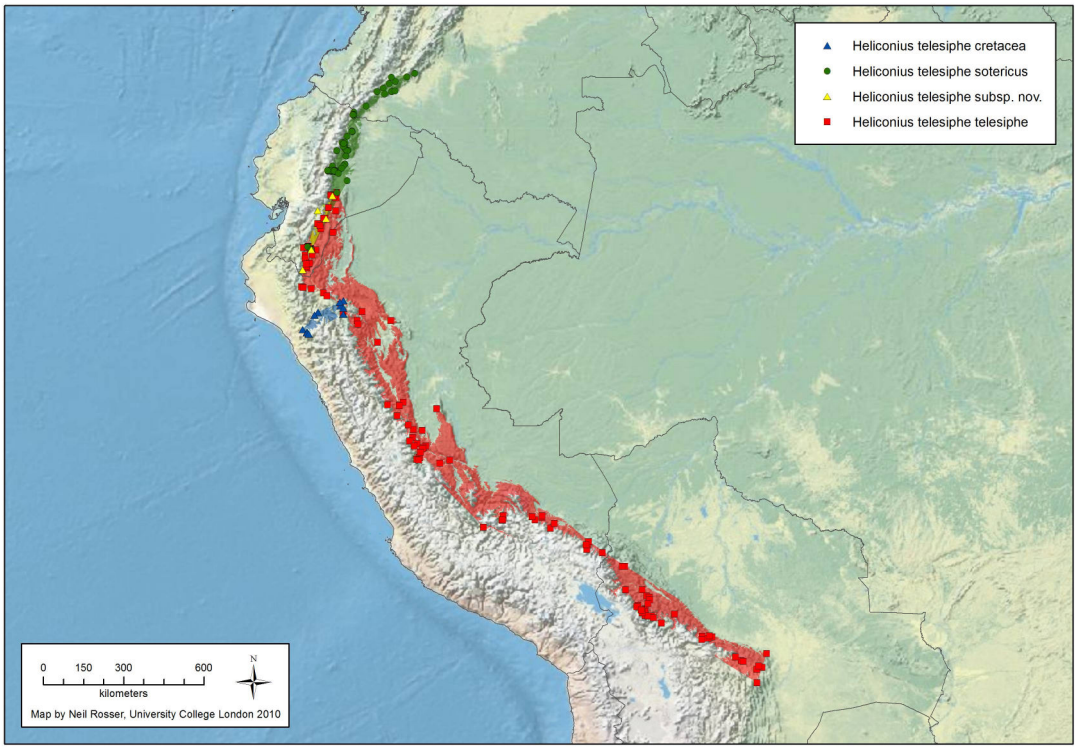

Heliconius numata lyrcaeus
Heliconius numata arcuella
Heliconius numata euphone
Heliconius numata aurora
Heliconius numata geminatus
0 150 300 600
kilometers
N
Map by Neil Rosser, University College London 2010

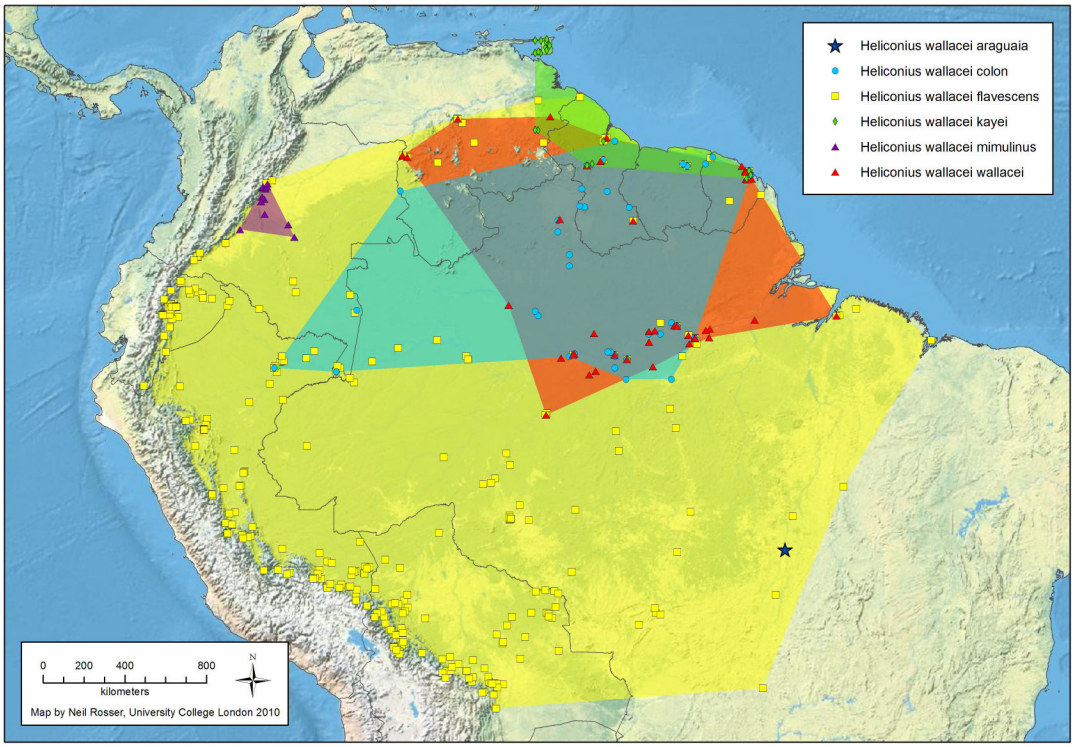

Heliconius pardalinus ariadne
Heliconius pardalinus butleri
Heliconius pardalinus dilatus
Heliconius pardalinus julia
Heliconius pardalinus lucescens
Heliconius pardalinus maeon
Heliconius pardalinus orteguaza
Heliconius pardalinus pardalinus
Heliconius pardalinus radiosus
Heliconius pardalinus sergestus
Heliconius pardalinus tithoreides
0 150 300 600
kilometers
N
Map by Neil Rosser, University College London 2010

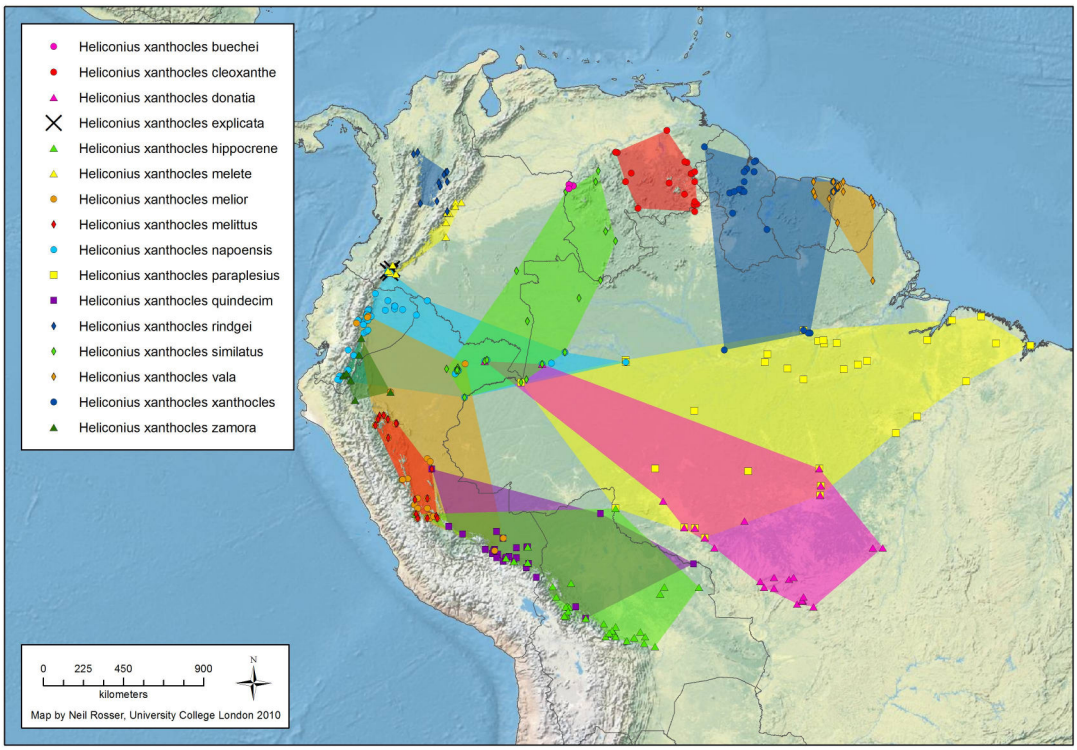

Heliconius ricini insulanus
Heliconius ricini ricini
0 200 400 800
kilometers
N
Map by Neil Rosser, University College London 2010

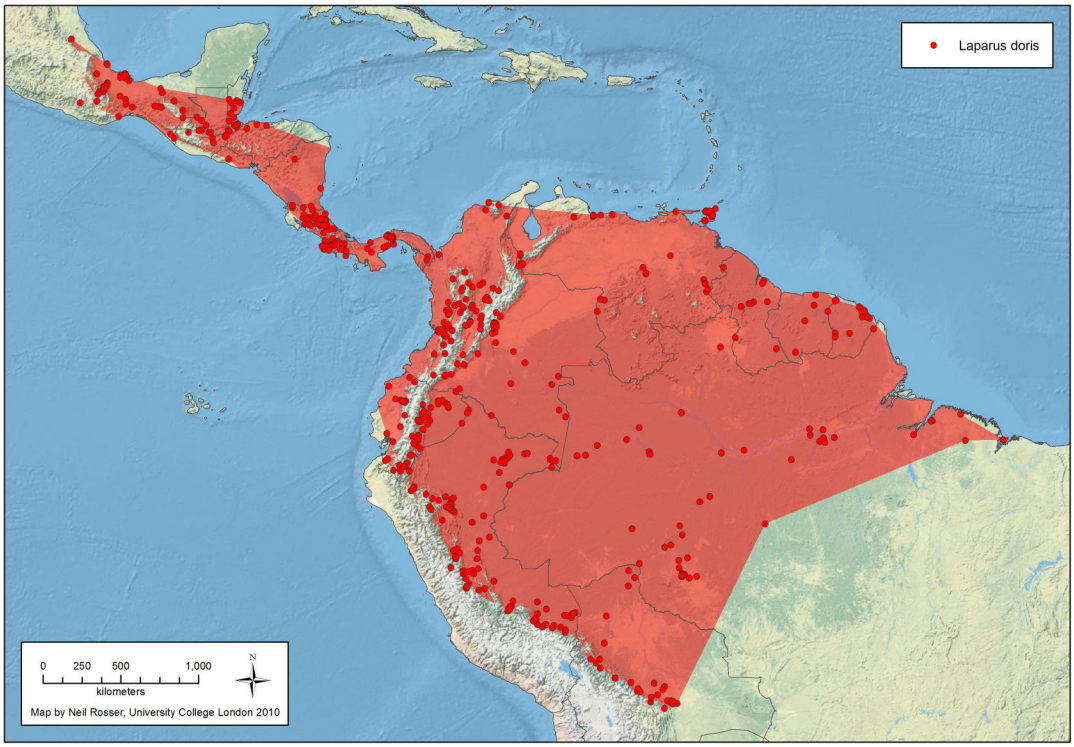

Heliconius hewitsoni
Heliconius leucadia leucadia
Heliconius leucadia pseudorhea
Heliconius sapho candidus
Heliconius sapho chocoensis
Heliconius sapho leuce
Heliconius sapho sapho
0 250 500 1,000
kilometers
N
Map by Neil Rosser, University College London 2010

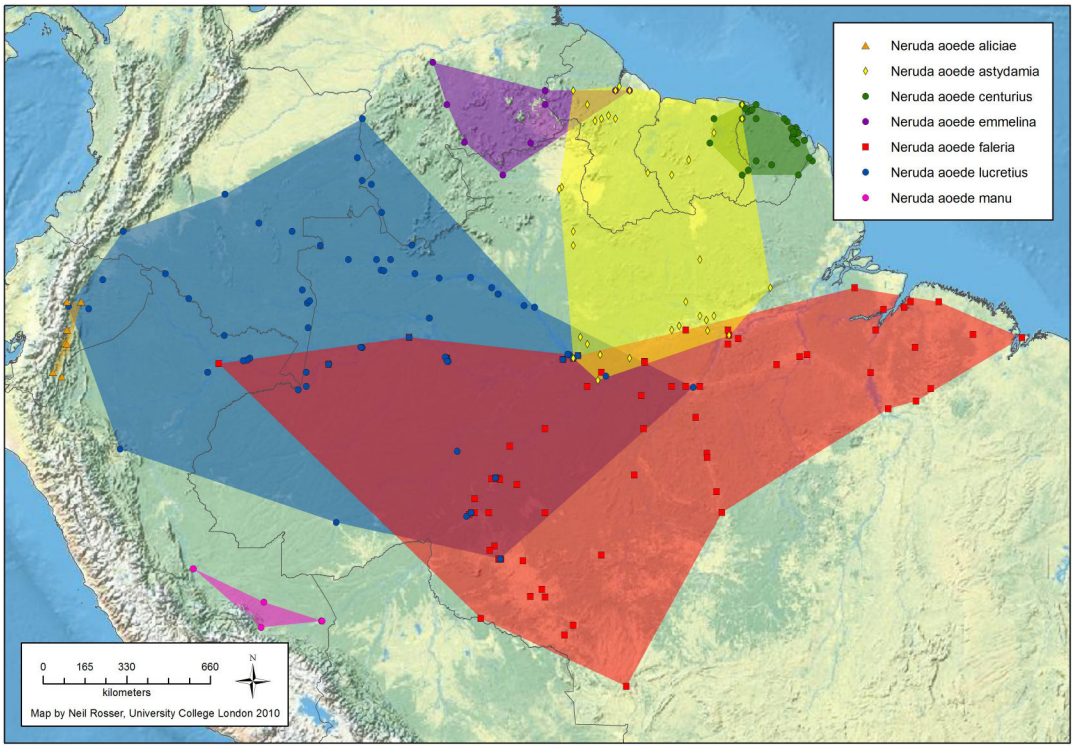

Heliconius sara apseudes
Heliconius sara brevimaculata
Heliconius sara elektra
Heliconius sara fulgidus
Heliconius sara magdalena
Heliconius sara sara
Heliconius sara sprucei
Heliconius sara theudela
Heliconius sara veraepacis
Heliconius sara williami
0 350 700 1,400
kilometers
N
Map by Neil Rosser, University College London 2010

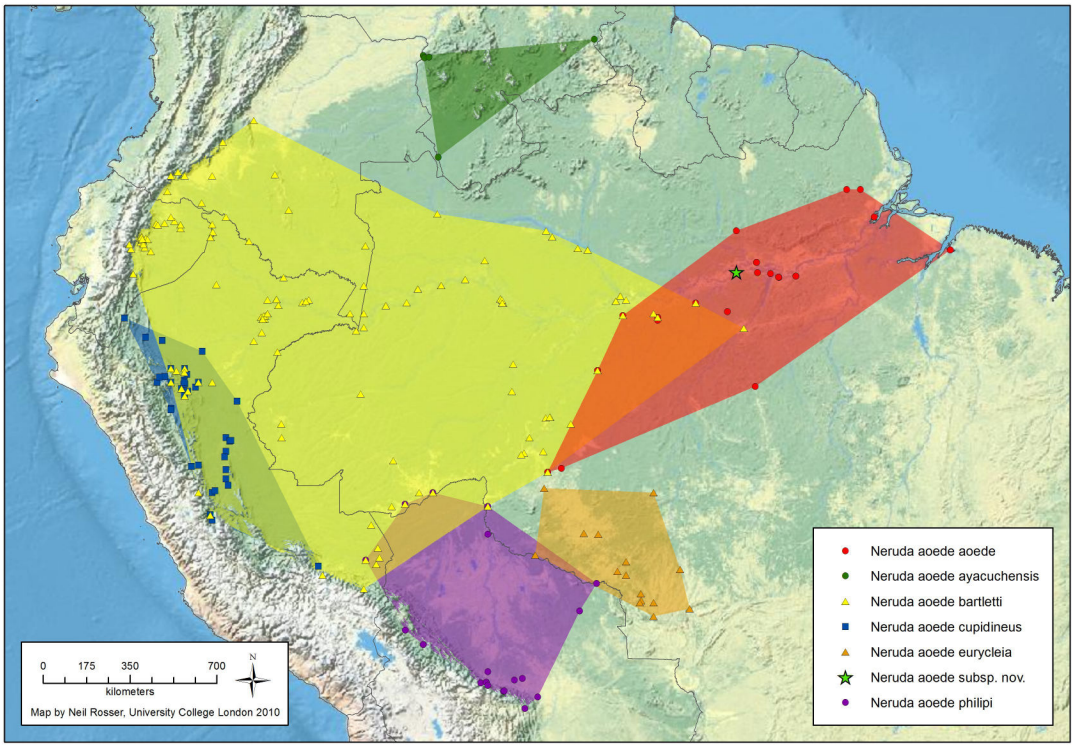

Heliconius telesiphe cretacea
Heliconius telesiphe sotericus
Heliconius telesiphe subsp. nov.
Heliconius telesiphe telesiphe
0 150 300 600
kilometers
N
Map by Neil Rosser, University College London 2010

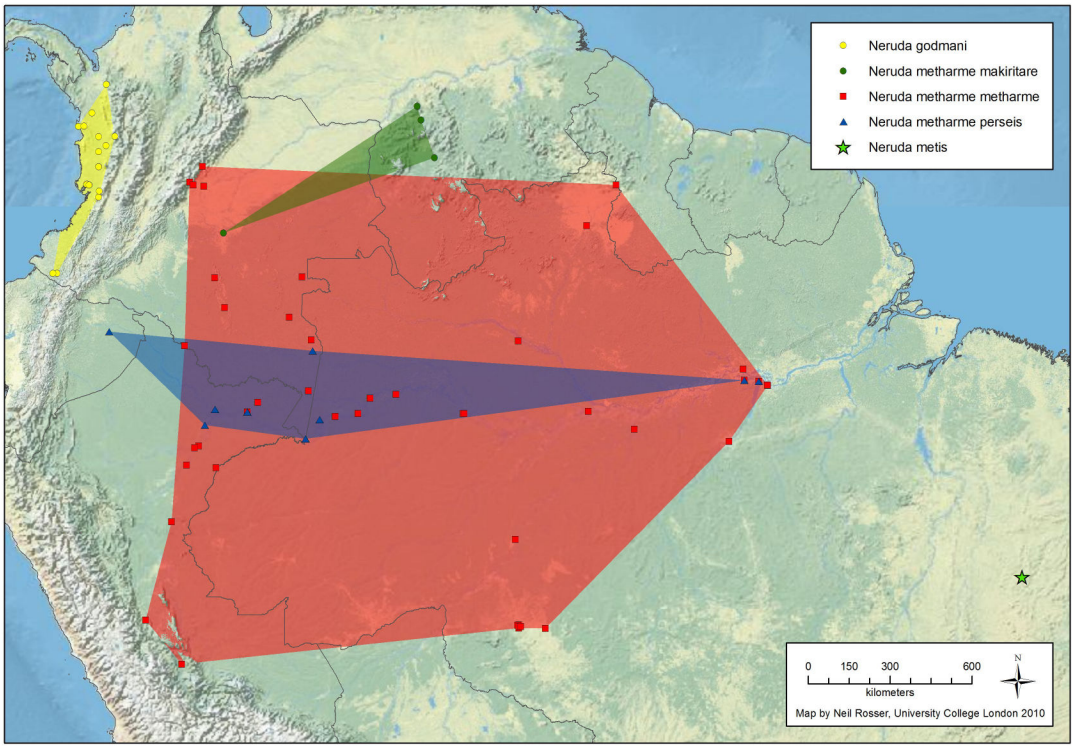

Heliconius wallacei araguaia
Heliconius wallacei colon
Heliconius wallacei flavescens
Heliconius wallacei kayei
Heliconius wallacei mimulinus
Heliconius wallacei wallacei
0 200 400 800
kilometers
N
Map by Neil Rosser, University College London 2010

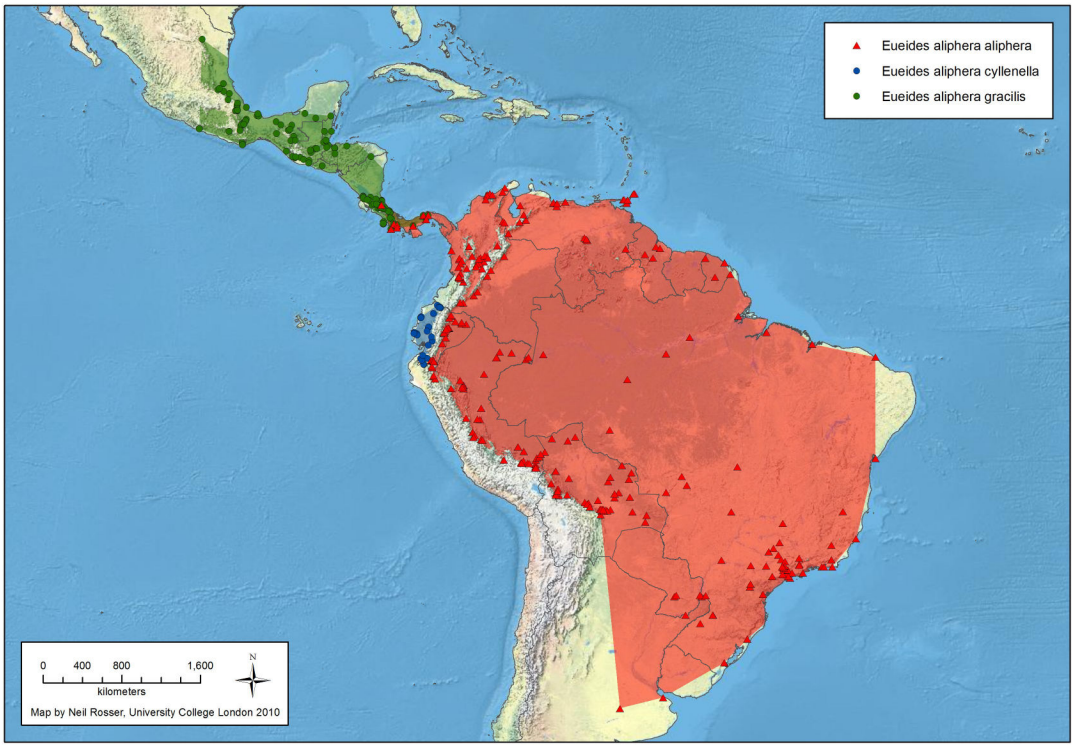

Heliconius xanthocles buechei
Heliconius xanthocles cleoxanthe
Heliconius xanthocles donatia
Heliconius xanthocles explicata
Heliconius xanthocles hippocrene
Heliconius xanthocles melete
Heliconius xanthocles melior
Heliconius xanthocles melittus
Heliconius xanthocles napoensis
Heliconius xanthocles paraplesius
Heliconius xanthocles quindecim
Heliconius xanthocles rindgei
Heliconius xanthocles similatus
Heliconius xanthocles vala
Heliconius xanthocles xanthocles
Heliconius xanthocles zamora
0 225 450 900
kilometers
N
Map by Neil Rosser, University College London 2010

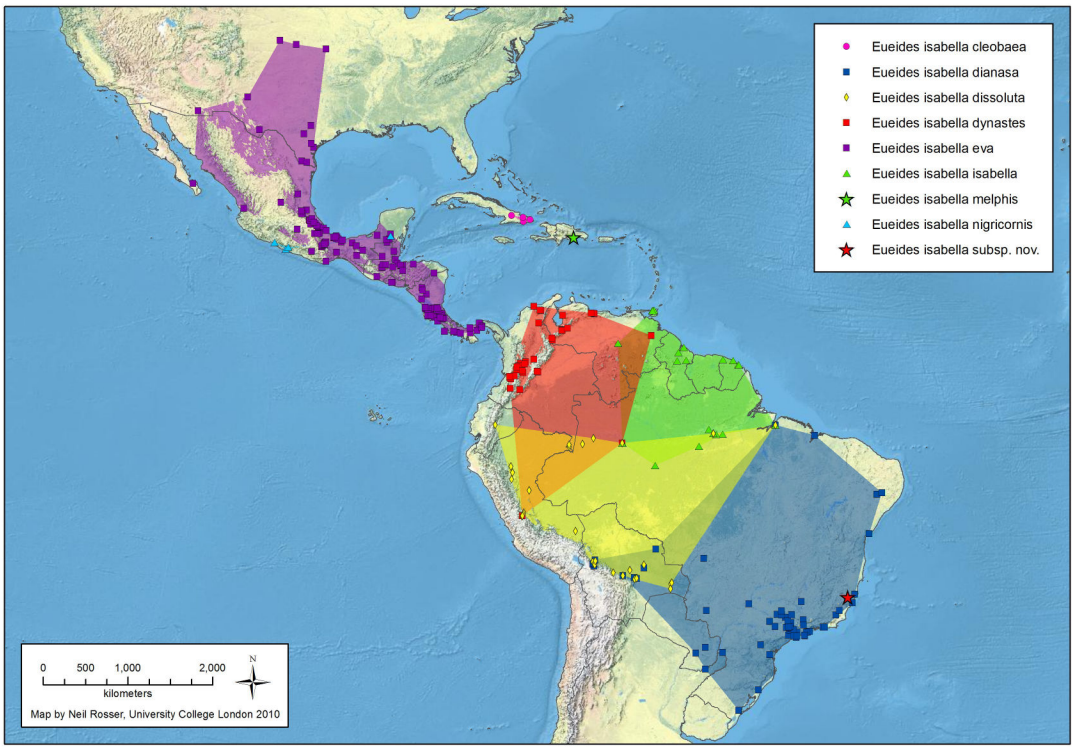

Laparus doris
0 250 500 1,000
kilometers
N
Map by Neil Rosser, University College London 2010

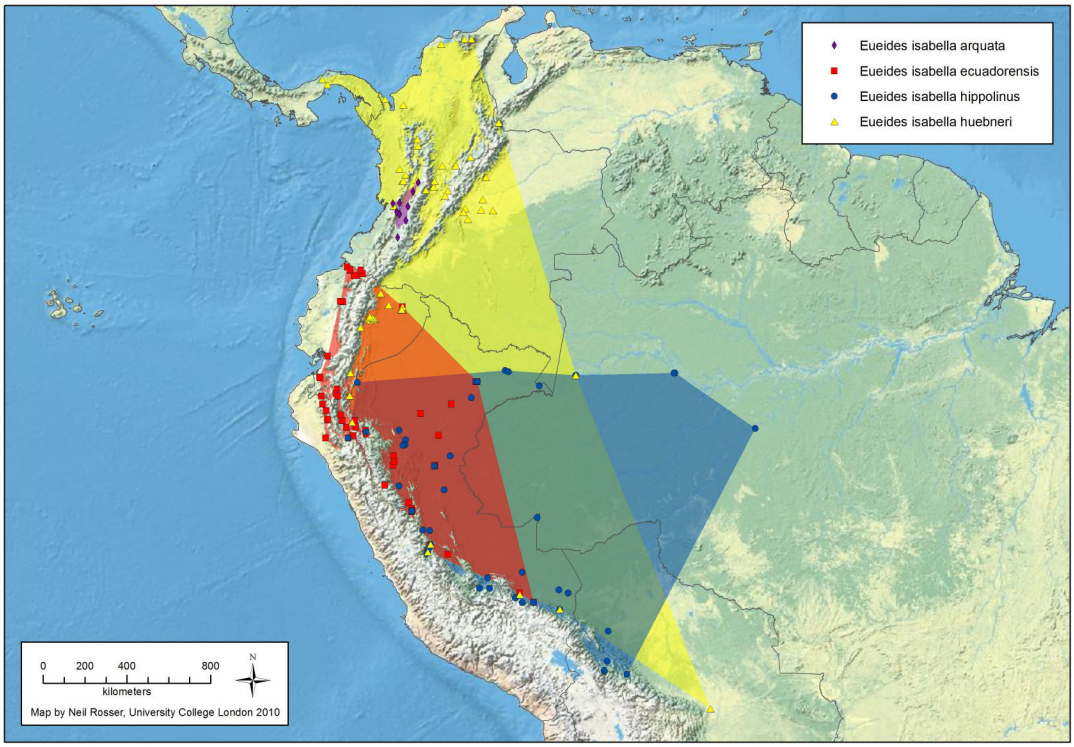

Neruda aoede aliciae
Neruda aoede astydamia
Neruda aoede centurius
Neruda aoede emmelina
Neruda aoede faleria
Neruda aoede lucretius
Neruda aoede manu
0 165 330 660
kilometers
N
Map by Neil Rosser, University College London 2010

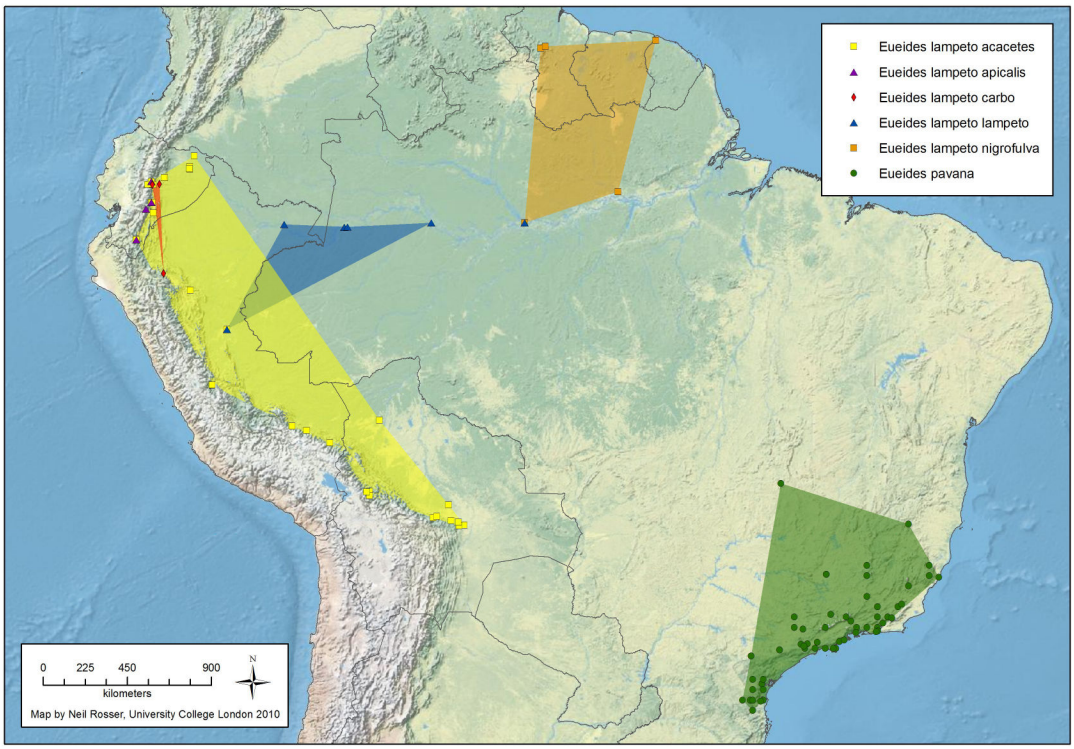

Neruda aoede aoede
Neruda aoede ayacuchensis
Neruda aoede bartletti
Neruda aoede cupidineus
Neruda aoede eurycleia
Neruda aoede subsp. nov.
Neruda aoede philipi
0 175 350 700
kilometers
N
Map by Neil Rosser, University College London 2010

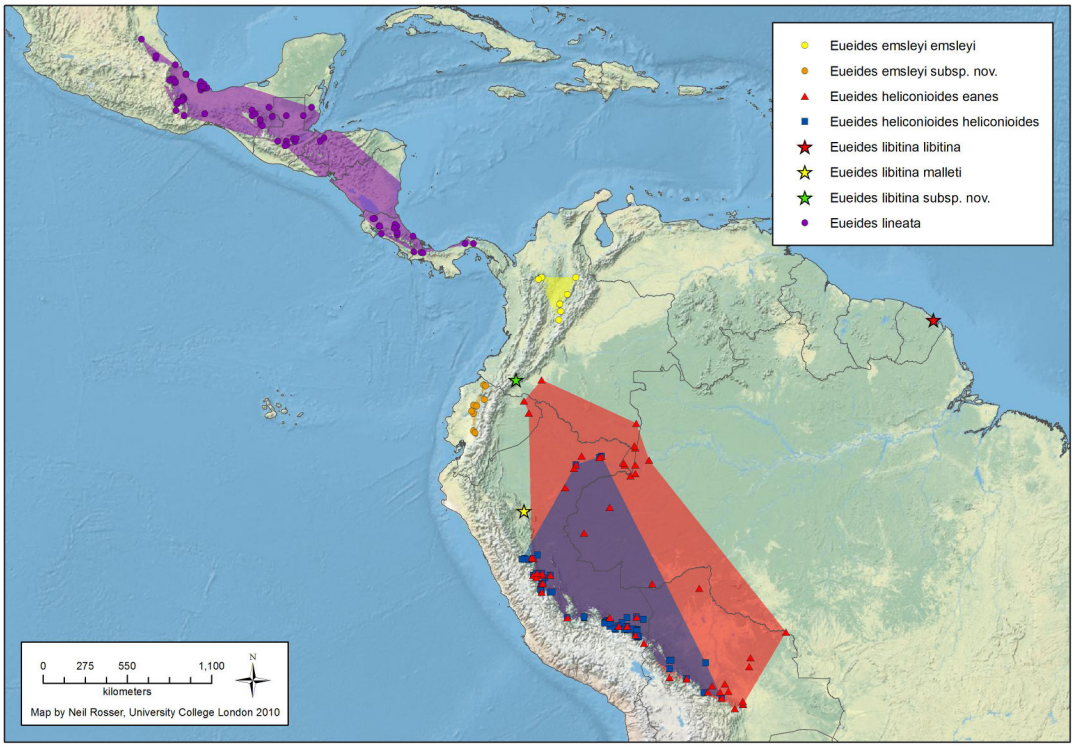

Neruda godmani
Neruda metharme makiritare
Neruda metharme metharme
Neruda metharme perseis
Neruda metis
0 150 300 600
kilometers
N
Map by Neil Rosser, University College London 2010

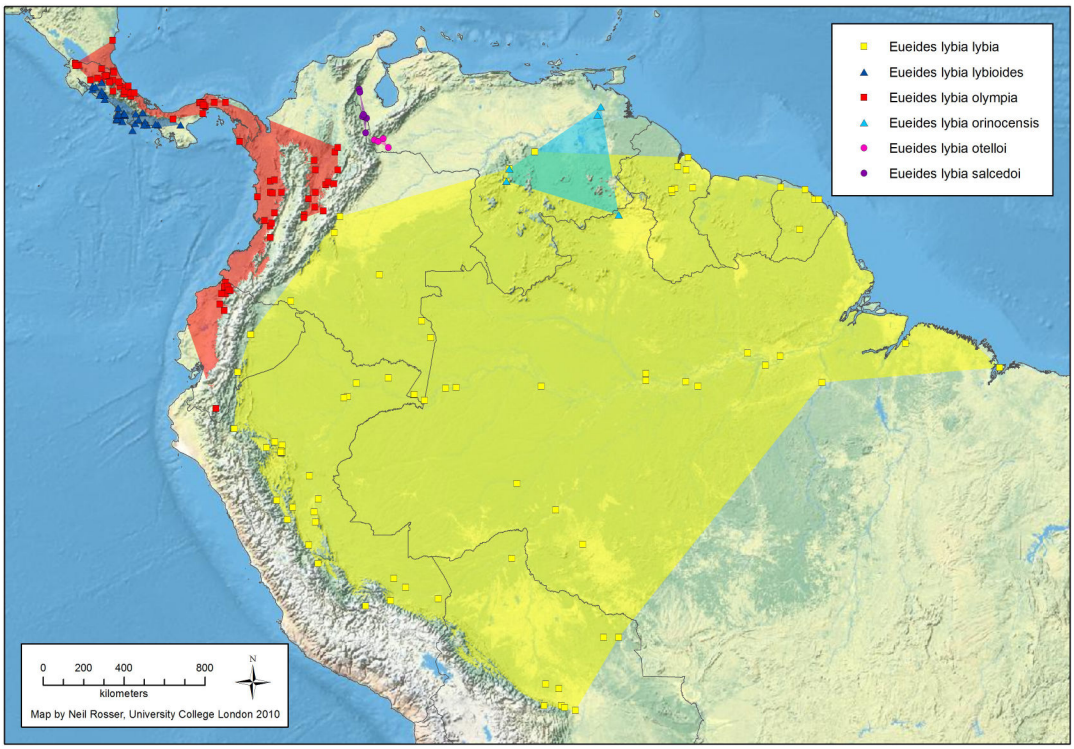

Eueides aliphera aliphera
Eueides aliphera cyllenella
Eueides aliphera gracilis
0 400 800 1,600
kilometers
N
Map by Neil Rosser, University College London 2010

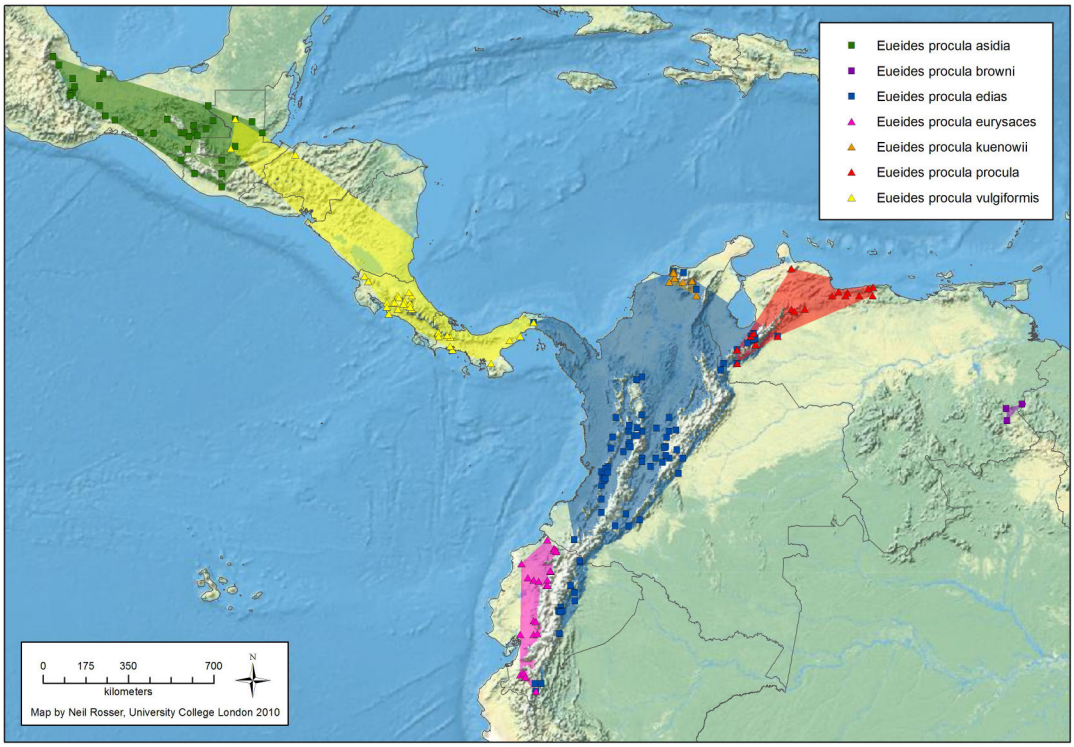

Eueides isabella cleobaea
Eueides isabella dianasa
Eueides isabella dissoluta
Eueides isabella dynastes
Eueides isabella eva
Eueides isabella isabella
Eueides isabella melphis
Eueides isabella nigricornis
Eueides isabella subsp. nov.
0 500 1,000 2,000
kilometers
N
Map by Neil Rosser, University College London 2010

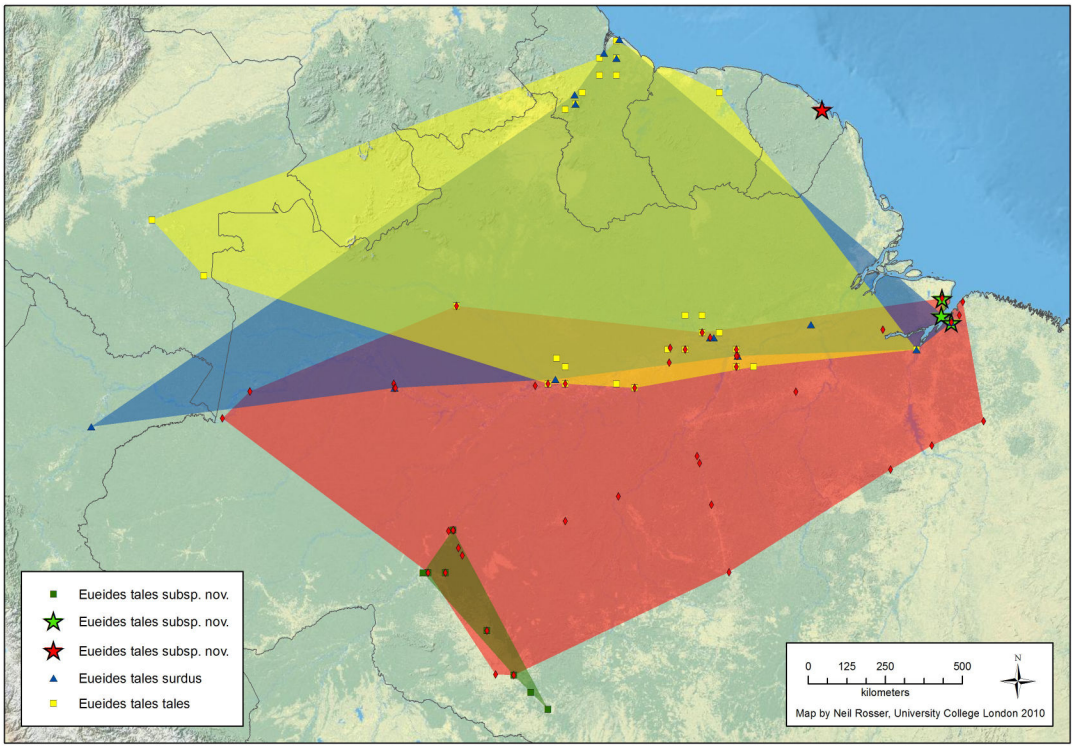

Eueides isabella arquata
Eueides isabella ecuadorensis
Eueides isabella hippolinus
Eueides isabella huebneri
0 200 400 800
kilometers
N
Map by Neil Rosser, University College London 2010

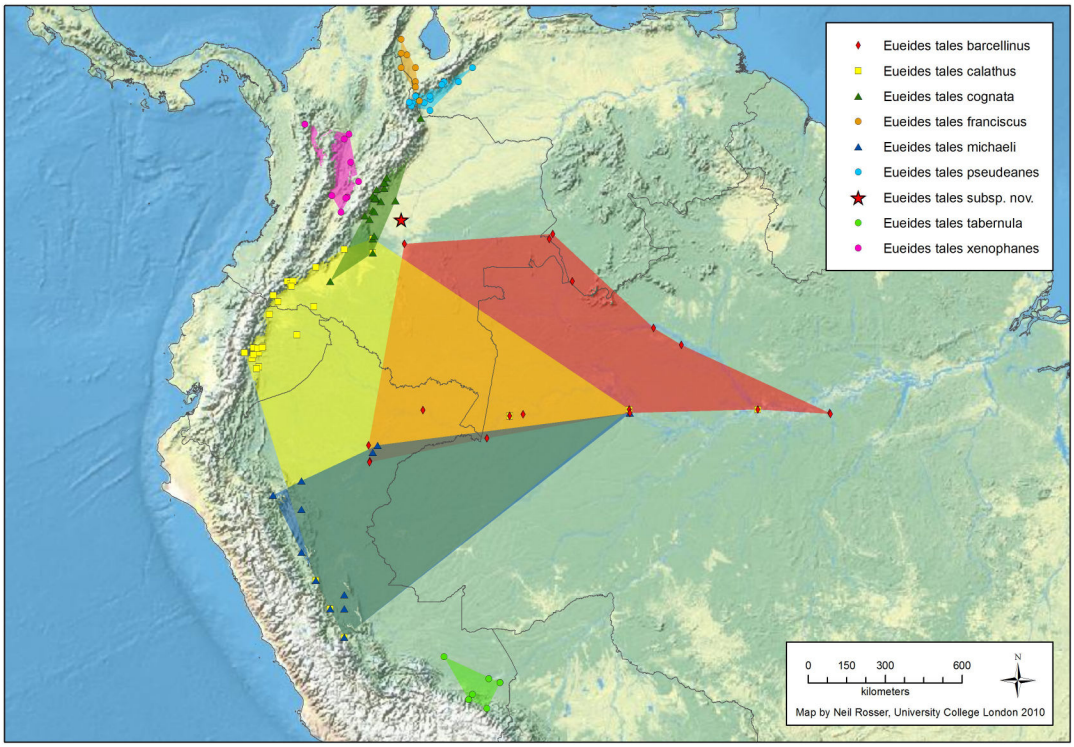

Eueides lampeto acacetes
Eueides lampeto apicalis
Eueides lampeto carbo
Eueides lampeto lampeto
Eueides lampeto nigrofulva
Eueides pavana
0 225 450 900
kilometers
N
Map by Neil Rosser, University College London 2010

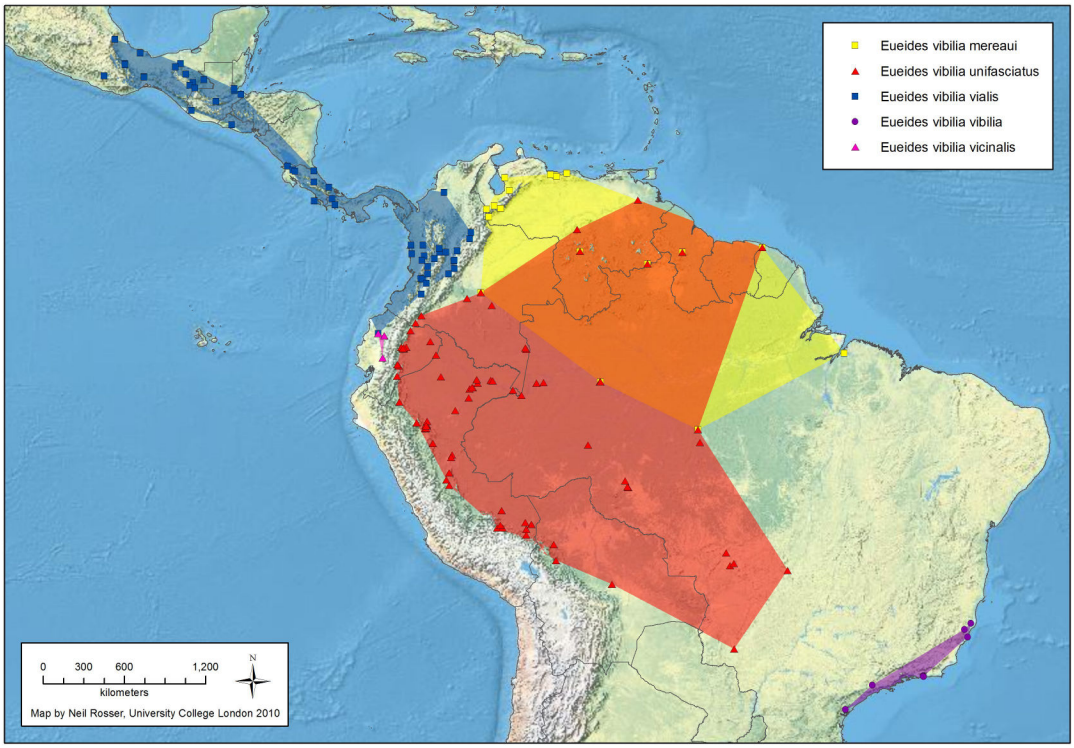

Eueides emsleyi emsleyi
Eueides emsleyi subsp. nov.
Eueides heliconioides eanes
Eueides heliconioides heliconioides
Eueides libitina libitina
Eueides libitina malleti
Eueides libitina subsp. nov.
Eueides lineata
0 275 550 1,100
kilometers
N
Map by Neil Rosser, University College London 2010

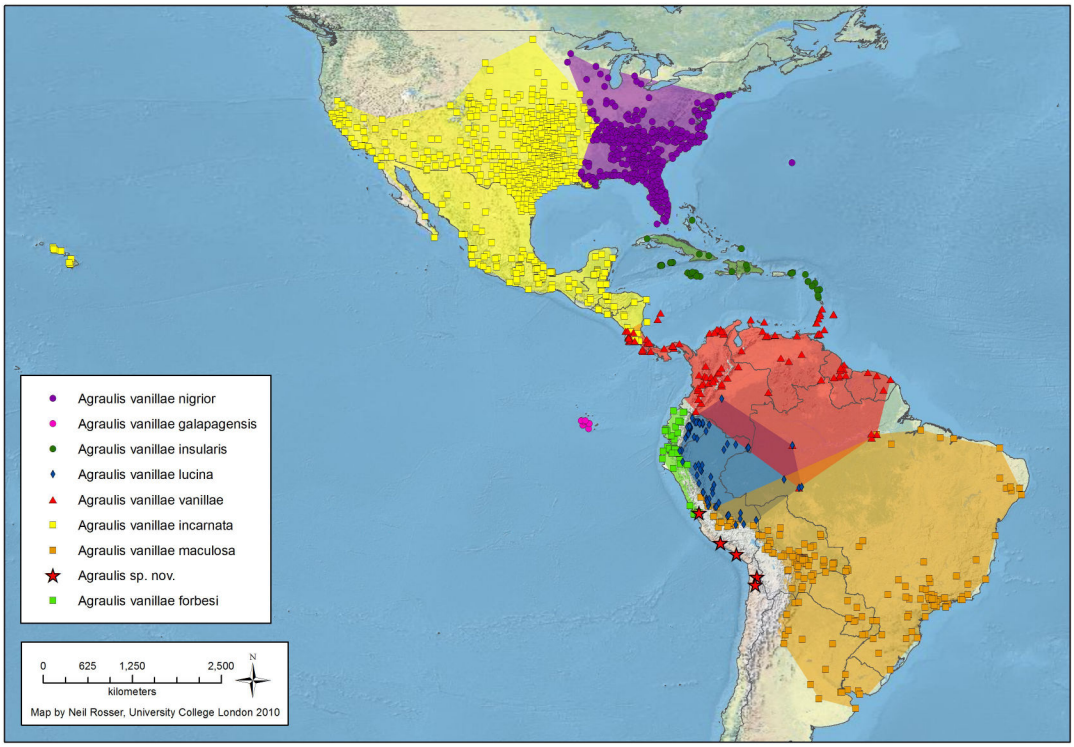

Eueides lybia lybia
Eueides lybia lybioides
Eueides lybia olympia
Eueides lybia orinocensis
Eueides lybia otelloi
Eueides lybia salcedoi
0 200 400 800
kilometers
N
Map by Neil Rosser, University College London 2010

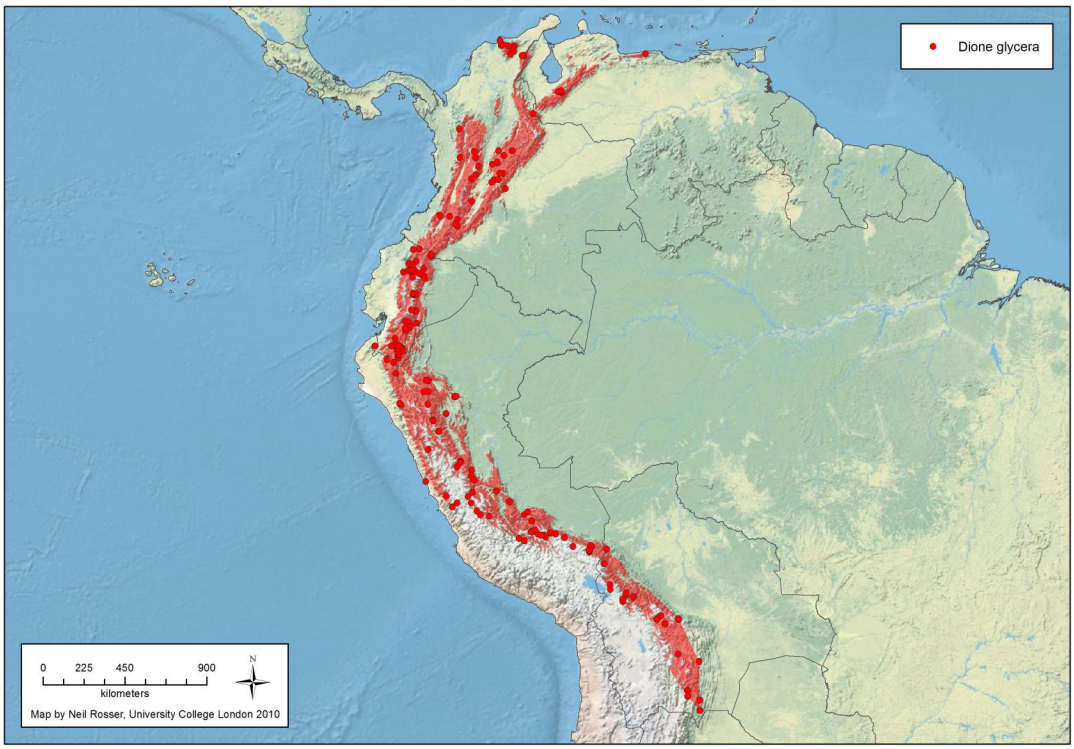

Eueides procula asidia
Eueides procula browni
Eueides procula edias
Eueides procula eurysaces
Eueides procula kuenowii
Eueides procula procula
Eueides procula vulgiformis
0 175 350 700
kilometers
N
Map by Neil Rosser, University College London 2010

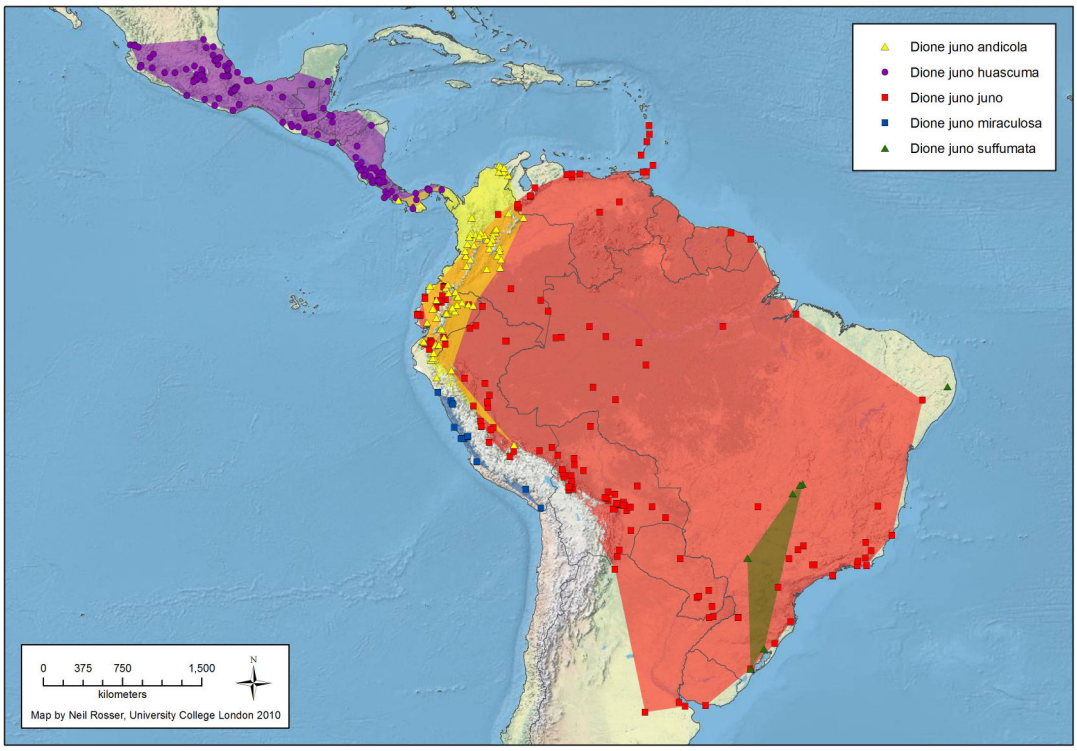

Eueides tales subsp. nov.
Eueides tales subsp. nov.
Eueides tales subsp. nov.
Eueides tales surdus
Eueides tales tales
0 125 250 500
kilometers
N
Map by Neil Rosser, University College London 2010

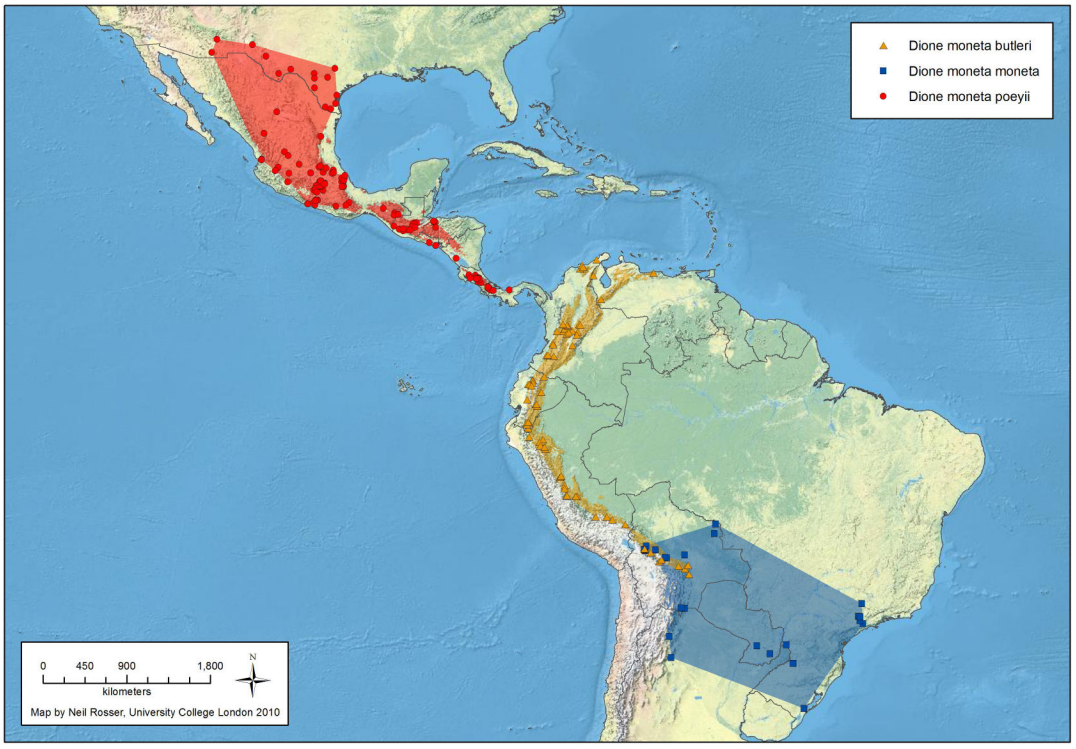

Eueides tales barcellinus
Eueides tales calathus
Eueides tales cognata
Eueides tales franciscus
Eueides tales michaeli
Eueides tales pseudeanes
Eueides tales subsp. nov.
Eueides tales tabernula
Eueides tales xenophanes
0 150 300 600
kilometers
N
Map by Neil Rosser, University College London 2010

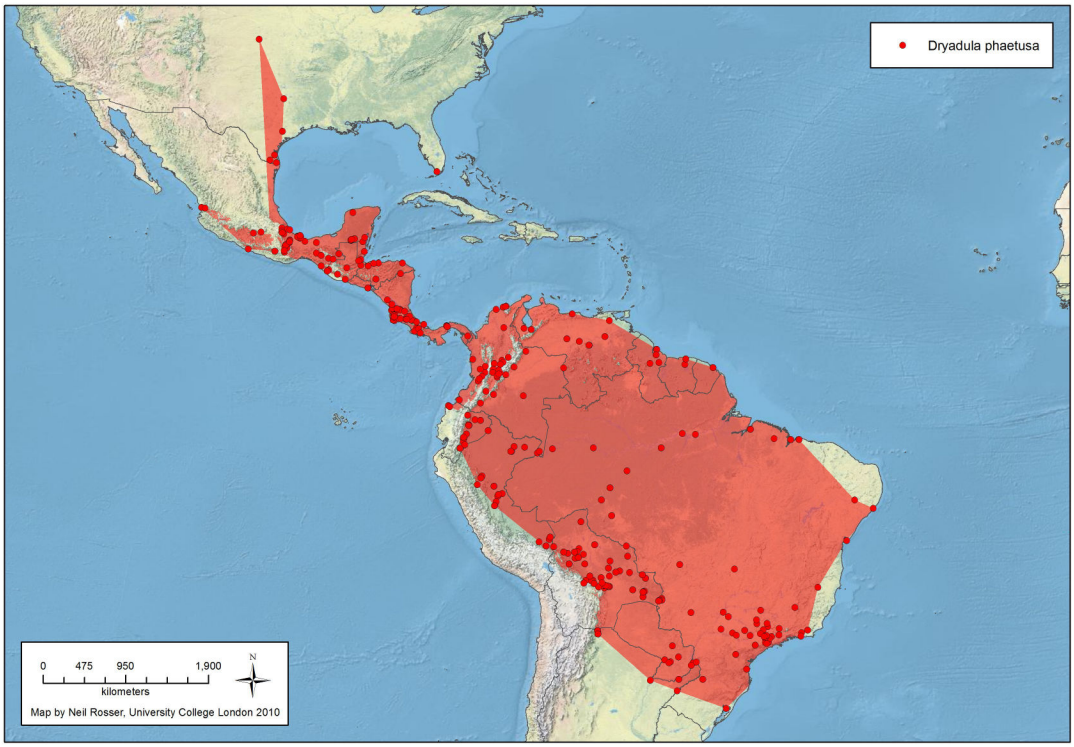

Eueides vibilia mereaui
Eueides vibilia unifasciatus
Eueides vibilia vialis
Eueides vibilia vibilia
Eueides vibilia vicinalis
0 300 600 1,200
kilometers
N
Map by Neil Rosser, University College London 2010

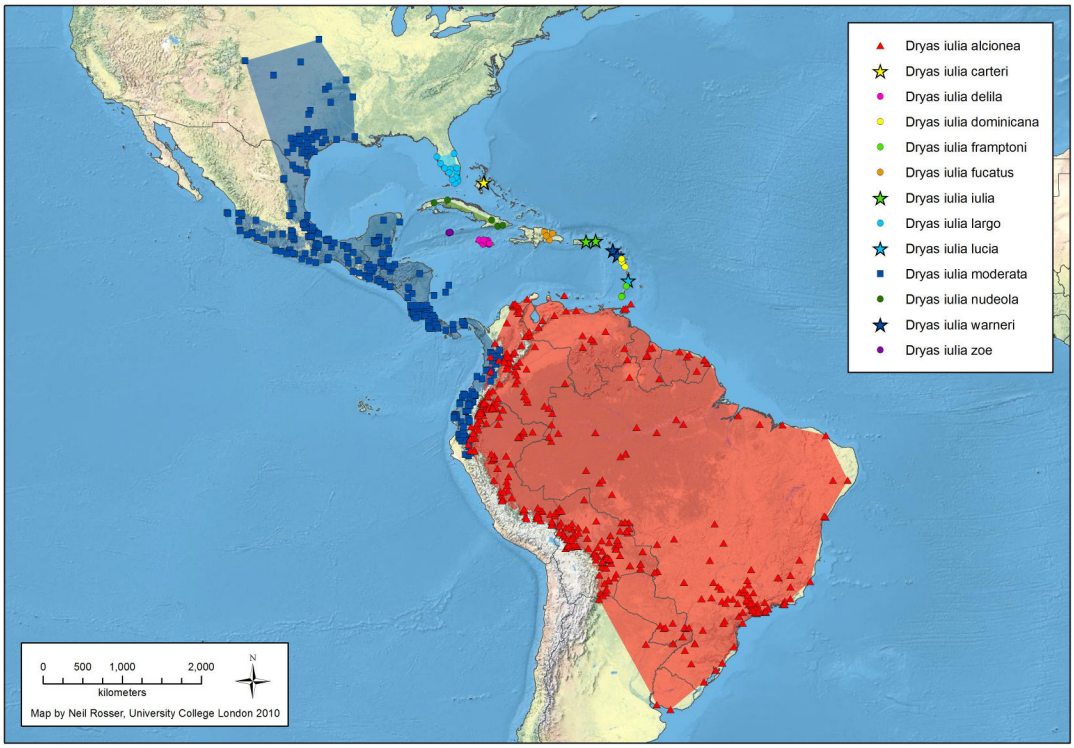

Agraulis vanillae nigrior
Agraulis vanillae galapagensis
Agraulis vanillae insularis
Agraulis vanillae lucina
Agraulis vanillae vanillae
Agraulis vanillae incarnata
Agraulis vanillae maculosa
Agraulis sp. nov.
Agraulis vanillae forbesi
0 625 1,250 2,500
kilometers
N
Map by Neil Rosser, University College London 2010

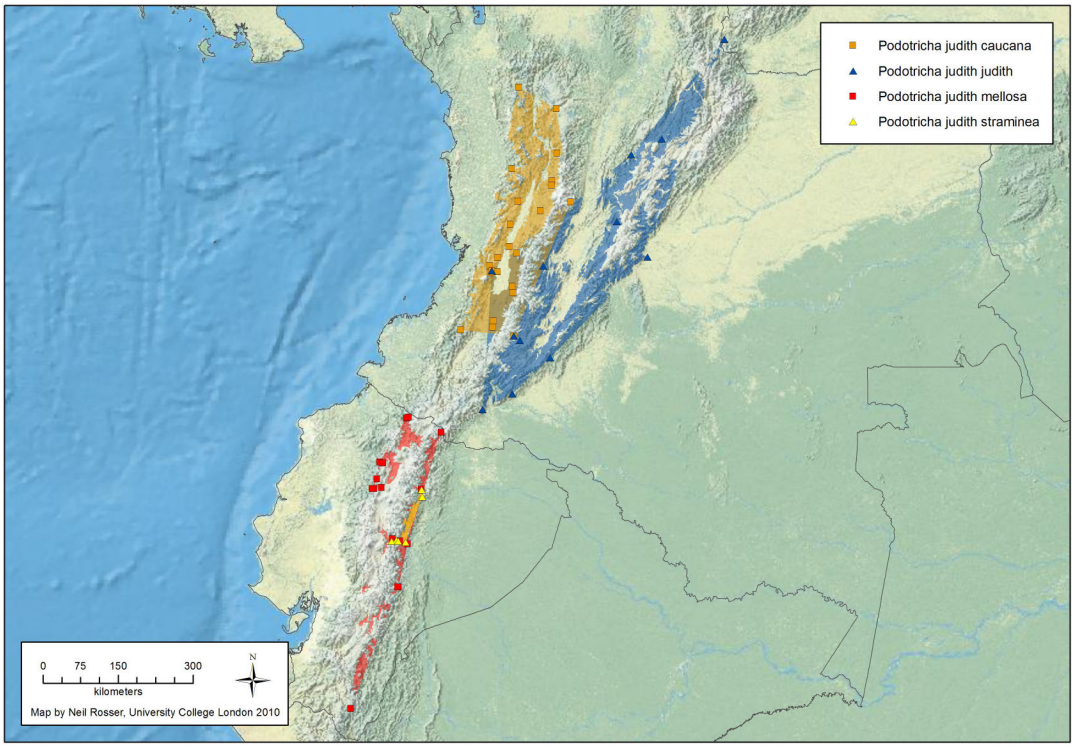

Dione glycera
0 225 450 900
kilometers
N
Map by Neil Rosser, University College London 2010


Dione juno andicola
Dione juno huascuma
Dione juno juno
Dione juno miraculosa
Dione juno suffumata
0 375 750 1,500
kilometers
N
Map by Neil Rosser, University College London 2010


Dione moneta butleri
Dione moneta moneta
Dione moneta poeyii
0 450 900 1,800
kilometers
N
Map by Neil Rosser, University College London 2010


Dryadula phaetusa
0 475 950 1,900
kilometers
N
Map by Neil Rosser, University College London 2010


Dryas iulia alcionea
Dryas iulia carteri
Dryas iulia delila
Dryas iulia dominicana
Dryas iulia framptoni
Dryas iulia fucatus
Dryas iulia iulia
Dryas iulia largo
Dryas iulia lucia
Dryas iulia moderata
Dryas iulia nudeola
Dryas iulia warneri
Dryas iulia zoe
0 500 1,000 2,000
kilometers
N
Map by Neil Rosser, University College London 2010



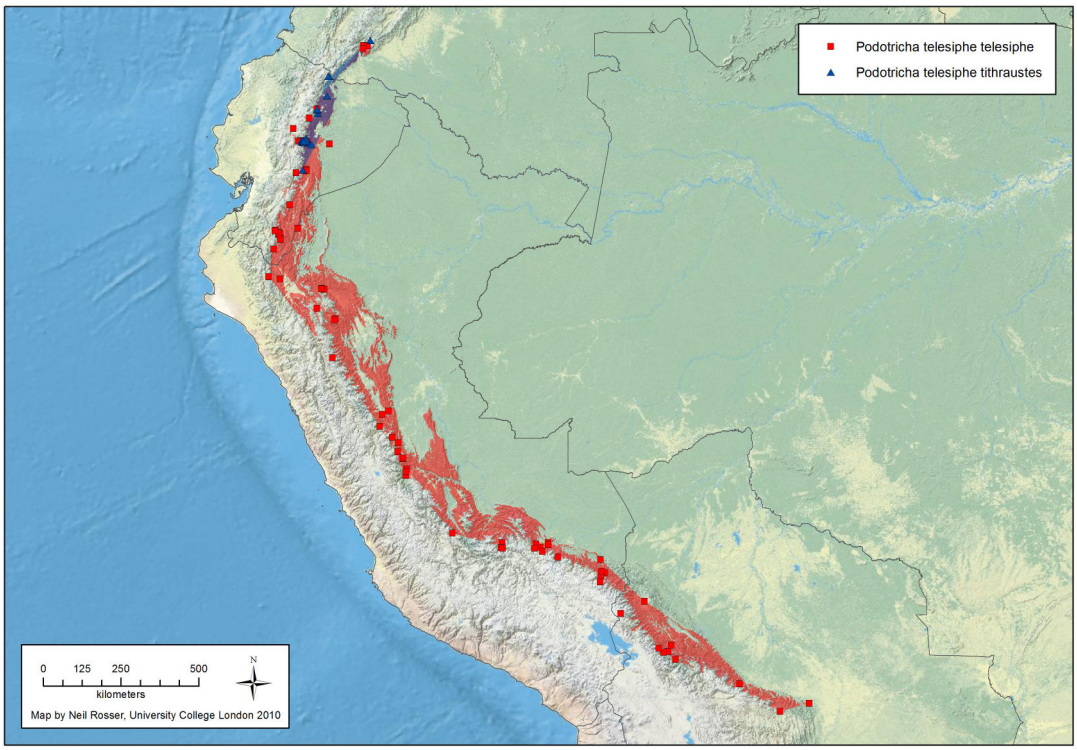


Figure A.1.1. Maps of species and subspecies ranges.

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Appendix 2.

Table A.2.1. Relaxed biological species where different from Lamas (2004).

Taxon	**Reference**
Heliconius chestertonii	Arias et al. (2008)
Heliconius eratosignis	Dasmahapatra et al. (in prep.)
Heliconis pachinus	
Heliconius hewitsoni	

Table A.2.2. Relaxed biological species sister comparisons

Agraulis sp. nov. vs *Agraulis vanillae*
Dione glycera vs *Dione juno*
Dryas iulia vs *Dryadula phaetusa*
Eueides isabella vs *Eueides lineata*
Eueides lampeto vs *Eueides vibilia*
Eueides lybia vs *Eueides tales*
Heliconiues hierax vs *Heliconius xanthocles*
Heliconius atthis vs *Heliconius hecale*
Heliconius burneyi vs *Heliconius wallacei*
Heliconius charithonia vs *Heliconius peruvianus*
Heliconius chestertonii vs *Heliconius erato*
Heliconius clysonymus vs *Heliconius telesiphe*
Heliconius congener vs *Heliconus eleuchia*
Heliconius cydno vs *Heliconius heurippa*
Heliconius demeter vs *Heliconius eratosignis*
Heliconius elevatus vs *Heliconius pardalinus*
Heliconius ethilla vs *Heliconius nattereri*
Heliconius ismenius vs *Heliconius numata*
Heliconius leucadia vs *Heliconius sara*
Heliconius pachinus vs *Heliconius timareta*
Neruda aoede vs *Neruda metharme*
Philaethria dido vs *Philaethria ostara (*cf. *diatonica)*
Podotricha judith vs *Podotricha telesiphe*

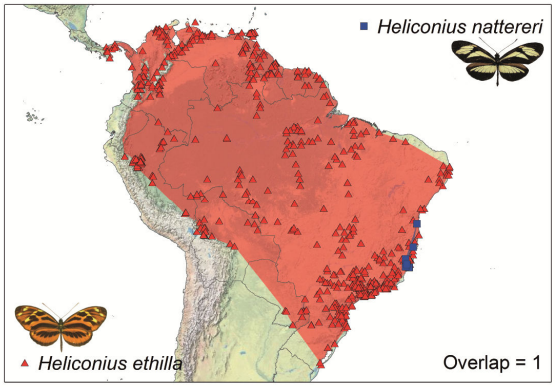
Table A.2.3. Strict biological species where different from Lamas (2004).

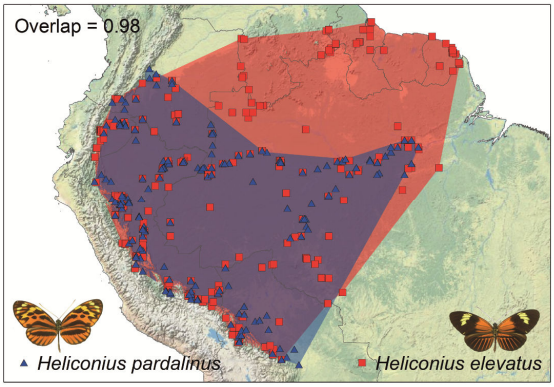
Taxon	Synonyms	Reference
Heliconius erato	*Heliconius chestertonii* *Heliconius himera*	
Heliconius cydno	*Heliconius heurippa* *Heliconius pachinus* *Heliconius timareta* *Heliconius tristero*	
Heliconius charithonia	*Heliconius peruvianus*	
Heliconius sapho	*Heliconius hewitsoni*	
Agraulis sp. nov. (Peru)		Lamas, unpublished data.
Heliconius eratosignis		Dasmahapatra et al. (in prep.)

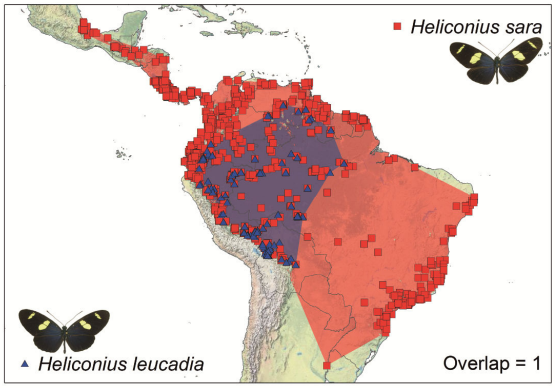
Table A.2.4. Strict biological species sister comparisons

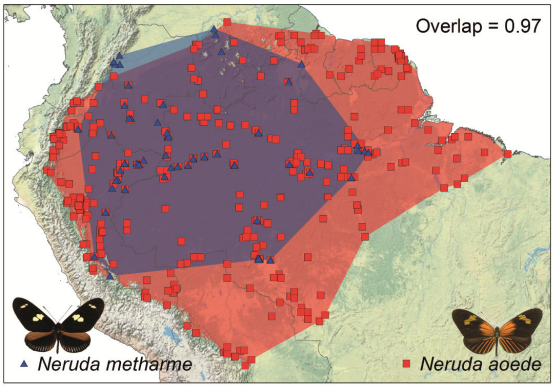
Agraulis sp. nov. vs *Agraulis vanillae*
Dione glycera vs *Dione juno*
Dryas iulia vs *Dryadula phaetusa*
Eueides isabella vs *Eueides lineata*
Eueides lampeto vs *Eueides vibilia*
Eueides lybia vs *Eueides tales*
Heliconiues hierax vs *Heliconius xanthocles*
Heliconius atthis vs *Heliconius hecale*
Heliconius burneyi vs *Heliconius wallacei*
Heliconius erato vs *Heliconius hermathena*
Heliconius clysonymus vs *Heliconius telesiphe*
Heliconius congener vs *Heliconus eleuchia*
Heliconius cydno vs *Heliconius melpomene*
Heliconius demeter vs *Heliconius eratosignis*
Heliconius elevatus vs *Heliconius pardalinus*
Heliconius ethilla vs *Heliconius nattereri*
Heliconius ismenius vs *Heliconius numata*
Heliconius leucadia vs *Heliconius sara*
Neruda aoede vs *Neruda metharme*

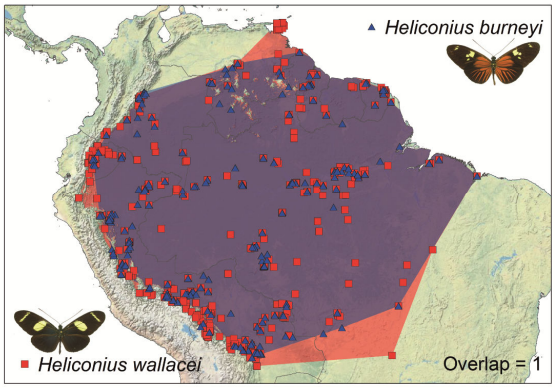
Philaethria dido vs *Philaethria ostara (*cf. *diatonica)*
Podotricha judith vs *Podotricha telesiphe*

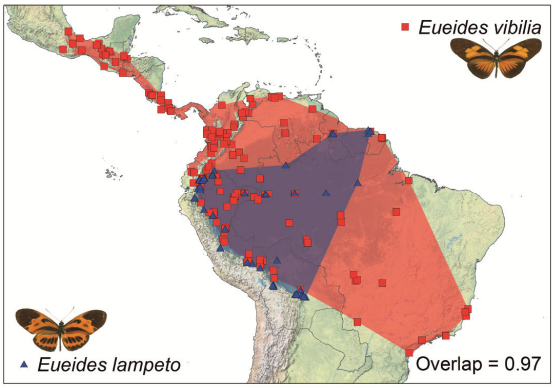

Heliconius nattereri
Heliconius ethilla
Overlap = 1

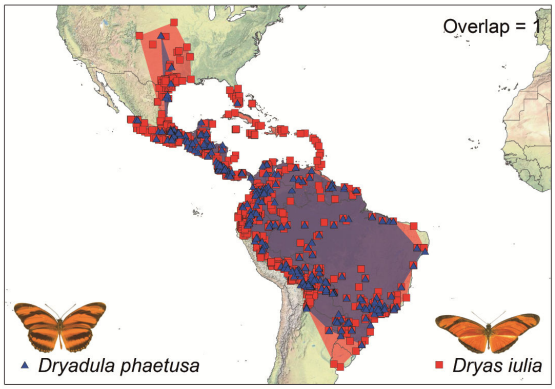

Overlap = 0.98
Heliconius pardalinus
Heliconius elevatus

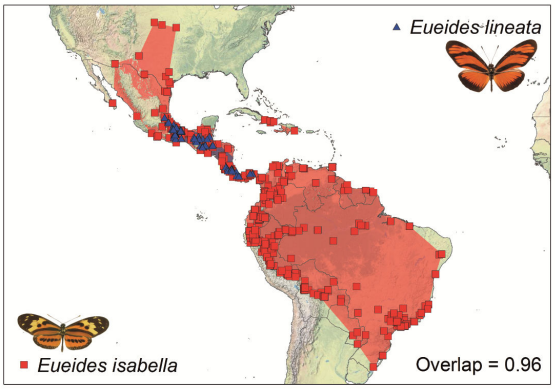

Heliconius sara
Heliconius leucadia
Overlap = 1

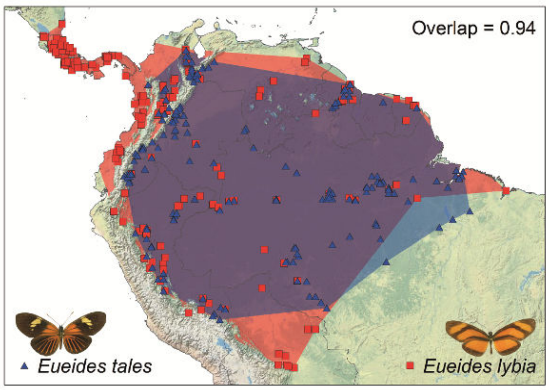

Overlap = 0.97
Neruda metharme
Neruda aoede

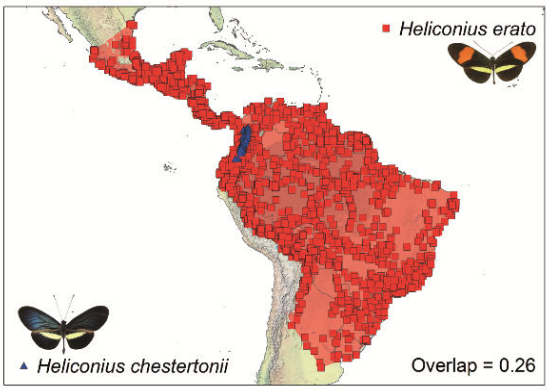

Heliconius burneyi
Heliconius wallacei
Overlap = 1

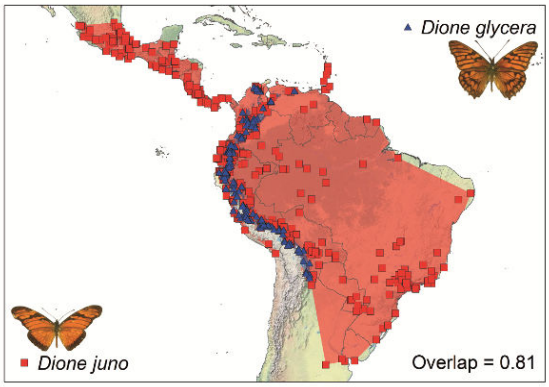

Eueides vibilia
Eueides lampeto
Overlap = 0.97

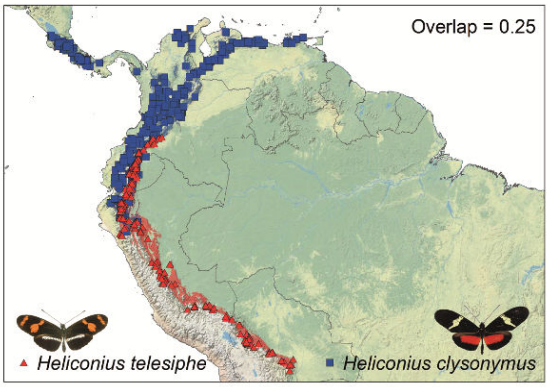

Overlap = 1
Dryadula phaetusa
Dryas iulia

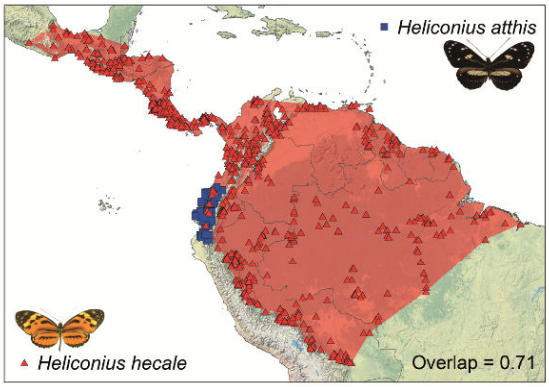

Eueides lineata
Eueides isabella
Overlap = 0.96

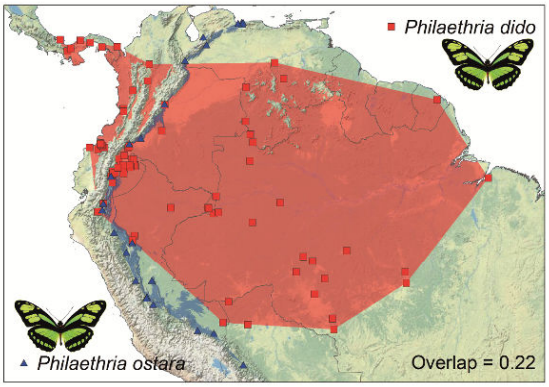

Overlap = 0.94
Eueides tales
Eueides lybia


Heliconius erato
Heliconius chestertonii
Overlap = 0.26


Dione glycera
Dione juno
Overlap = 0.81


Overlap = 0.25
Heliconius telesiphe
Heliconius clysonymus


Heliconius atthis
Heliconius hecale
Overlap = 0.71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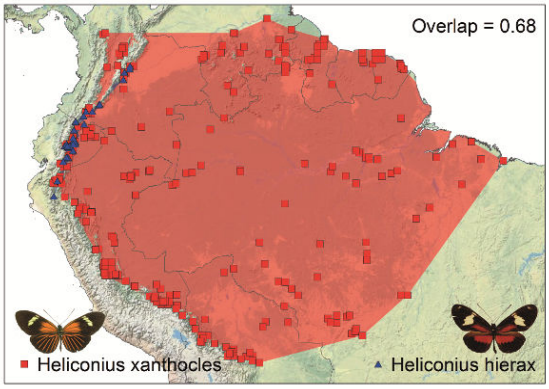
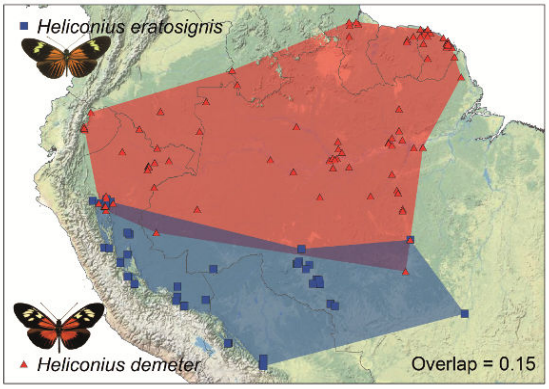
Philaethria ostara
Overlap = 0.22


Overlap = 0.68
Heliconius xanthocles
Heliconius hierax


Heliconius erafosignis
Heliconius demeter
Overlap = 0.15

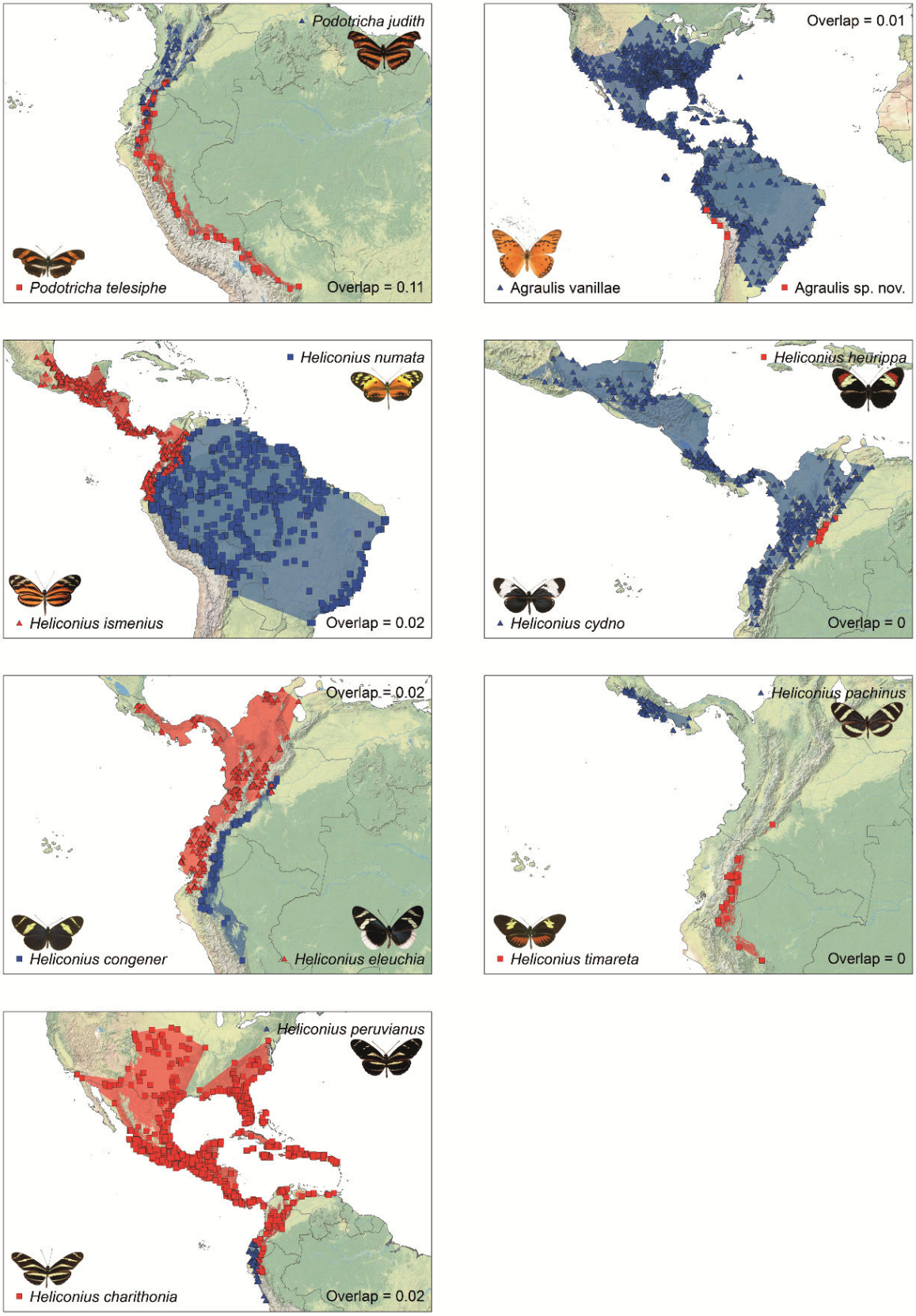


Figure A.2.1 Maps of range overlap between heliconiine sister species (relaxed BSC).

Figure A.2.5. Colour pattern classification for sister species of heliconiines. Only heliconiine species used in the present study are included. Key to colour patterns: 1: dennis rayed, 2: red on forewing (yellow on hindwing), 3: red on hindwing, yellow on forewing, 4: tiger, 5: *Heliconius heurippa*, 6: *Elzunia*, 7:*Heliconius hecalesia*, 8: *Actinote*, 9: *Heliconius telesiphe cretecea*, 10: *Laparus doris* (green morph), 11: blue and yellow forewing, 12: white or yellow on hindwing & usually forewing, 13: black with yellow bars 1, 14: black with yellow bars 2, 15: *Podotricha judith*, 16: orange, 17: green.

	Wing colour pattern																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Agraulis sp. nov.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Agraulis vanillae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Dione glycera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Dione juno	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Dione moneta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Dryadula phaetusa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Dryas iulia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Eueides aliphera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Eueides emsleyi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Eueides heliconioides	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Eueides isabella	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Eueides lampeto	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Eueides lineata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Eueides lybia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Eueides pavana	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Eueides procula	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Eueides tales	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eueides vibilia	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Heliconius antiochus	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
Heliconius astraea	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heliconius atthis	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Heliconius besckei	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heliconius burneyi	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Heliconius charithonia	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Heliconius chestertonii	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Heliconius clysonymus	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heliconius congener	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Heliconius cydno	0	0	0	0	0	1	1	0	0	0	1	1	0	0	0	0	0
Heliconius demeter	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heliconius egeria	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heliconius eleuchia	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Heliconius elevatus	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heliconius erato	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heliconius eratosignis	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heliconius ethilla	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Heliconius hecale	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Heliconius hecalesia	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0
Heliconius hecuba	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0
Heliconius hermathena	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heliconius heurippa	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Heliconius hewitsoni	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Heliconius hierax	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heliconius himera	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heliconius hortense	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heliconius ismenius	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Heliconius lalitae	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heliconius leucadia	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
Heliconius luciana	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0
Heliconius melpomene	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heliconius nattereri	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0
Heliconius numata	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Heliconius pachinus	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Heliconius pardalinus	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Heliconius peruvianus	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Heliconius ricini	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heliconius sapho	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0

Heliconius sara	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0
Heliconius telesiphe	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Heliconius timareta	1	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Heliconius tristero	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heliconius wallacei	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Heliconius xanthocles	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Laparus doris	1	0	1	0	0	0	0	0	0	1	1	0	1	0	0	0	0
Neruda aoede	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Neruda godmani	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Neruda metharme	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Philaethria dido	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Philaethria ostara	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Podotricha judith	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Podotricha telesiphe	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table A.2.6. Parameter values for simulations of relaxed biological species where the observed data frequently arose ($p \geq 0.05$ for all three indices). CO = complete overlap, ZO = zero overlap.

Sympatric speciation events	Starting range size	Range movements	Range growth	Allopatric mode of speciation	Median cases of CO	Probability of observed CO	Median cases of ZO	Probability of observed ZO	Median bimodality	Probability of observed bimodality
8	1309	0.25	0	parapatric	5	0.074	7	0.622	0.273	0.450
8	1595	0.25	0	parapatric	5	0.054	8	0.566	0.273	0.460
8	1595	0.25	0.1	dispersal	5	0.054	10	0.056	0.364	0.968
8	3190	0.25	0	parapatric	6	0.142	8	0.544	0.318	0.638
8	3190	0.25	0.1	dispersal	6	0.126	7	0.780	0.273	0.438
9	654	0.25	0	parapatric	5	0.074	7	0.780	0.242	0.292
9	1309	0.25	0	parapatric	6	0.194	7	0.770	0.273	0.486
9	1309	0.25	0.1	dispersal	6	0.158	10	0.056	0.409	0.820
9	1595	0.25	0	parapatric	6	0.246	7	0.784	0.303	0.580
9	1595	0.25	0.1	dispersal	6	0.222	9	0.130	0.379	0.942
9	3190	0.25	0	parapatric	6	0.438	7	0.794	0.318	0.754
9	3190	0.25	0.1	dispersal	6	0.394	6	1.000	0.303	0.562
9	3190	0.5	0	parapatric	5	0.106	7	0.806	0.265	0.358
9	3190	0.5	0	vicariance	5	0.092	10	0.084	0.341	0.882
9	3190	0.5	0.1	dispersal	5	0.126	7	0.914	0.250	0.260
9	3190	0.75	0.1	dispersal	4	0.052	7	0.780	0.227	0.192
10	654	0.25	0	parapatric	5	0.184	7	0.976	0.265	0.370
10	1309	0.25	0	parapatric	6	0.406	6	1.000	0.303	0.508
10	1309	0.25	0.1	dispersal	6	0.436	9	0.132	0.417	0.740
10	1309	0.5	0	parapatric	5	0.084	6	1.000	0.212	0.170
10	1309	0.5	0.1	dispersal	5	0.072	9	0.124	0.303	0.636
10	1595	0.25	0	parapatric	6	0.472	7	0.994	0.303	0.600
10	1595	0.25	0.1	dispersal	6	0.500	9	0.206	0.409	0.774
10	1595	0.5	0	parapatric	5	0.082	6	1.000	0.220	0.230
10	1595	0.5	0	vicariance	5	0.066	9	0.210	0.303	0.596
10	1595	0.5	0.1	dispersal	5	0.104	9	0.220	0.303	0.650
10	3190	0.25	0	parapatric	7	0.856	7	0.984	0.364	0.846
10	3190	0.25	0.1	dispersal	7	0.834	6	1.000	0.318	0.586
10	3190	0.5	0	parapatric	6	0.196	6	1.000	0.265	0.406
10	3190	0.5	0	vicariance	6	0.250	9	0.178	0.364	0.974
10	3190	0.5	0.1	dispersal	6	0.252	6	1.000	0.265	0.298
10	3190	0.75	0	parapatric	5	0.050	6	1.000	0.220	0.184
10	3190	0.75	0	vicariance	5	0.072	8	0.416	0.273	0.394
10	3190	0.75	0.1	dispersal	5	0.092	6	1.000	0.227	0.184
10	3190	1	0.1	dispersal	4	0.054	7	0.938	0.205	0.134
11	654	0.25	0	parapatric	6	0.316	6	1.000	0.254	0.340
11	654	0.25	0.1	dispersal	6	0.388	9	0.056	0.417	0.732
11	1309	0.25	0	parapatric	7	0.660	6	1.000	0.303	0.554
11	1309	0.25	0.1	dispersal	7	0.652	8	0.220	0.424	0.640
11	1309	0.5	0	parapatric	5	0.132	6	1.000	0.212	0.186
11	1309	0.5	0	vicariance	5	0.078	8	0.356	0.273	0.482
11	1309	0.5	0.1	dispersal	5	0.154	8	0.260	0.303	0.646
11	1595	0.25	0	parapatric	7	0.748	6	1.000	0.303	0.568
11	1595	0.25	0.1	dispersal	7	0.804	8	0.426	0.409	0.796
11	1595	0.5	0	parapatric	5	0.190	6	1.000	0.227	0.204
11	1595	0.5	0	vicariance	5	0.176	8	0.374	0.303	0.560
11	1595	0.5	0.1	dispersal	5	0.224	8	0.314	0.318	0.650
11	3190	0.25	0	parapatric	8	1.000	6	1.000	0.364	0.892
11	3190	0.25	0.1	dispersal	8	1.000	6	1.000	0.318	0.634
11	3190	0.5	0	parapatric	6	0.446	6	1.000	0.265	0.402
11	3190	0.5	0	vicariance	6	0.386	8	0.316	0.364	0.928
11	3190	0.5	0.1	dispersal	6	0.472	6	1.000	0.265	0.294

11	3190	0.75	0	parapatric	5	0.148	6	1.000	0.227	0.182
11	3190	0.75	0	vicariance	5	0.122	7	0.612	0.265	0.412
11	3190	0.75	0.1	dispersal	5	0.206	6	1.000	0.227	0.222
11	3190	1	0	parapatric	4	0.052	6	1.000	0.182	0.076
11	3190	1	0	dispersal	4	0.052	10	0.050	0.295	0.522
11	3190	1	0.1	dispersal	4	0.072	6	1.000	0.189	0.116
12	654	0.25	0	parapatric	7	0.580	5	0.982	0.265	0.352
12	654	0.25	0	vicariance	6	0.548	9	0.124	0.417	0.714
12	654	0.25	0.1	dispersal	7	0.568	9	0.156	0.409	0.746
12	654	0.5	0	parapatric	4	0.054	6	1.000	0.182	0.052
12	654	0.5	0.1	dispersal	4	0.078	9	0.134	0.273	0.484
12	1309	0.25	0	parapatric	7	0.992	5	0.980	0.303	0.534
12	1309	0.25	0	vicariance	8	1.000	9	0.114	0.485	0.350
12	1309	0.25	0.1	dispersal	7	0.972	8	0.524	0.424	0.726
12	1309	0.5	0	parapatric	5	0.234	5	0.984	0.212	0.168
12	1309	0.5	0	vicariance	5	0.198	7	0.522	0.303	0.512
12	1309	0.5	0.1	dispersal	6	0.278	8	0.454	0.303	0.624
12	1309	0.75	0	parapatric	4	0.050	6	1.000	0.159	0.050
12	1309	0.75	0.1	dispersal	4	0.064	8	0.416	0.242	0.284
12	1595	0.25	0	parapatric	8	1.000	5	0.964	0.318	0.636
12	1595	0.25	0	vicariance	8	1.000	9	0.142	0.485	0.324
12	1595	0.25	0.1	dispersal	8	1.000	7	0.634	0.409	0.780
12	1595	0.5	0	parapatric	6	0.282	5	0.938	0.227	0.166
12	1595	0.5	0	vicariance	6	0.300	7	0.576	0.303	0.592
12	1595	0.5	0.1	dispersal	6	0.336	7	0.598	0.318	0.614
12	1595	0.75	0	parapatric	4	0.092	6	1.000	0.182	0.090
12	1595	0.75	0	vicariance	4	0.082	7	0.878	0.212	0.180
12	1595	0.75	0.1	dispersal	5	0.108	7	0.580	0.250	0.278
12	1595	1	0.1	dispersal	4	0.052	7	0.640	0.205	0.136
12	3190	0.25	0	parapatric	9	0.962	5	0.978	0.341	0.856
12	3190	0.25	0	vicariance	9	0.916	9	0.106	0.583	0.132
12	3190	0.25	0.1	dispersal	8	1.000	5	0.784	0.303	0.600
12	3190	0.5	0	parapatric	7	0.636	6	1.000	0.265	0.426
12	3190	0.5	0	vicariance	7	0.652	7	0.570	0.364	0.988
12	3190	0.5	0.1	dispersal	7	0.714	5	0.894	0.265	0.364
12	3190	0.75	0	parapatric	5	0.268	5	0.972	0.212	0.182
12	3190	0.75	0	vicariance	5	0.200	7	0.842	0.265	0.368
12	3190	0.75	0.1	dispersal	6	0.290	5	0.972	0.227	0.188
12	3190	1	0	parapatric	4	0.080	6	1.000	0.182	0.078
12	3190	1	0	vicariance	4	0.060	7	0.992	0.205	0.162
12	3190	1	0	dispersal	4	0.054	9	0.090	0.303	0.544
12	3190	1	0.1	dispersal	5	0.148	6	1.000	0.189	0.074
13	654	0.25	0	parapatric	7	0.828	5	0.778	0.265	0.404
13	654	0.25	0	vicariance	7	0.772	8	0.256	0.424	0.718
13	654	0.25	0.1	dispersal	7	0.848	8	0.378	0.409	0.834
13	654	0.5	0	parapatric	4	0.092	5	0.806	0.155	0.050
13	654	0.5	0	vicariance	5	0.106	7	0.758	0.227	0.230
13	654	0.5	0.1	dispersal	5	0.144	8	0.366	0.273	0.430
13	1309	0.25	0	parapatric	8	1.000	5	0.730	0.303	0.556
13	1309	0.25	0	vicariance	8	1.000	8	0.218	0.485	0.364
13	1309	0.25	0.1	dispersal	8	1.000	7	0.826	0.409	0.784
13	1309	0.5	0	parapatric	6	0.302	5	0.774	0.212	0.152
13	1309	0.5	0	vicariance	6	0.298	7	0.900	0.273	0.430
13	1309	0.5	0.1	dispersal	6	0.382	7	0.750	0.303	0.552
13	1309	0.75	0	vicariance	4	0.064	6	1.000	0.189	0.084
13	1309	0.75	0.1	dispersal	5	0.142	7	0.744	0.242	0.288

13	1595	0.25	0	parapatric	8	1.000	5	0.728	0.303	0.600
13	1595	0.25	0	vicariance	8	1.000	8	0.232	0.485	0.294
13	1595	0.25	0.1	dispersal	8	1.000	6	1.000	0.409	0.830
13	1595	0.5	0	parapatric	6	0.440	5	0.770	0.227	0.192
13	1595	0.5	0	vicariance	6	0.462	7	0.848	0.303	0.622
13	1595	0.5	0.1	dispersal	6	0.508	7	0.926	0.318	0.572
13	1595	0.75	0	parapatric	5	0.106	5	0.864	0.182	0.062
13	1595	0.75	0	vicariance	5	0.074	6	1.000	0.212	0.158
13	1595	0.75	0.1	dispersal	5	0.174	7	0.916	0.242	0.240
13	3190	0.25	0	parapatric	9	0.576	5	0.742	0.341	0.860
13	3190	0.25	0	vicariance	9	0.564	8	0.238	0.545	0.130
13	3190	0.25	0.1	dispersal	9	0.702	5	0.556	0.303	0.630
13	3190	0.5	0	parapatric	7	0.912	5	0.762	0.265	0.412
13	3190	0.5	0	vicariance	7	0.930	7	0.848	0.364	0.974
13	3190	0.5	0.1	dispersal	7	0.942	5	0.654	0.265	0.324
13	3190	0.75	0	parapatric	6	0.352	5	0.764	0.212	0.158
13	3190	0.75	0	vicariance	6	0.338	6	1.000	0.265	0.374
13	3190	0.75	0	dispersal	6	0.376	9	0.098	0.371	0.980
13	3190	0.75	0.1	dispersal	6	0.478	5	0.730	0.227	0.192
13	3190	1	0	parapatric	5	0.140	5	0.764	0.182	0.082
13	3190	1	0	vicariance	5	0.116	6	1.000	0.212	0.126
13	3190	1	0	dispersal	5	0.144	8	0.244	0.303	0.514
13	3190	1	0.1	dispersal	5	0.260	5	0.746	0.189	0.102
14	654	0.25	0	parapatric	8	1.000	4	0.502	0.242	0.284
14	654	0.25	0	vicariance	8	1.000	7	0.590	0.409	0.800
14	654	0.25	0.1	dispersal	8	1.000	7	0.672	0.409	0.846
14	654	0.5	0	parapatric	5	0.172	5	0.632	0.159	0.056
14	654	0.5	0	vicariance	5	0.142	6	1.000	0.227	0.162
14	654	0.5	0.1	dispersal	5	0.240	7	0.788	0.265	0.368
14	1309	0.25	0	parapatric	9	0.850	4	0.538	0.303	0.558
14	1309	0.25	0	vicariance	9	0.908	7	0.548	0.477	0.458
14	1309	0.25	0.1	dispersal	9	0.880	6	1.000	0.409	0.854
14	1309	0.5	0	parapatric	6	0.530	5	0.530	0.212	0.132
14	1309	0.5	0	vicariance	6	0.456	6	1.000	0.273	0.406
14	1309	0.5	0.1	dispersal	6	0.564	6	1.000	0.303	0.500
14	1309	0.75	0	vicariance	5	0.100	6	1.000	0.182	0.136
14	1309	0.75	0.1	dispersal	5	0.208	6	1.000	0.227	0.202
14	1309	1	0.1	dispersal	4	0.074	6	1.000	0.182	0.074
14	1595	0.25	0	parapatric	9	0.746	5	0.440	0.303	0.524
14	1595	0.25	0	vicariance	9	0.738	7	0.572	0.477	0.334
14	1595	0.25	0.1	dispersal	9	0.786	6	1.000	0.379	0.874
14	1595	0.5	0	parapatric	7	0.638	4	0.516	0.212	0.158
14	1595	0.5	0	vicariance	7	0.578	6	1.000	0.273	0.496
14	1595	0.5	0.1	dispersal	7	0.688	6	1.000	0.273	0.508
14	1595	0.75	0	vicariance	5	0.188	6	1.000	0.212	0.138
14	1595	0.75	0	dispersal	5	0.204	8	0.056	0.303	0.642
14	1595	0.75	0.1	dispersal	6	0.234	6	1.000	0.227	0.224
14	1595	1	0.1	dispersal	4	0.074	6	1.000	0.189	0.094
14	3190	0.25	0	parapatric	10	0.384	4	0.488	0.333	0.798
14	3190	0.25	0	vicariance	10	0.340	7	0.566	0.545	0.198
14	3190	0.25	0.1	dispersal	10	0.432	4	0.316	0.303	0.524
14	3190	0.5	0	parapatric	8	1.000	4	0.476	0.265	0.322
14	3190	0.5	0	vicariance	8	1.000	6	1.000	0.364	0.928
14	3190	0.5	0	dispersal	8	1.000	8	0.122	0.477	0.404
14	3190	0.5	0.1	dispersal	8	1.000	4	0.440	0.242	0.300
14	3190	0.75	0	parapatric	6	0.540	5	0.530	0.212	0.140

14	3190	0.75	0	vicariance	6	0.524	6	1.000	0.265	0.326
14	3190	0.75	0	dispersal	6	0.556	8	0.304	0.364	0.924
14	3190	0.75	0.1	dispersal	7	0.654	5	0.516	0.227	0.174
14	3190	1	0	parapatric	5	0.240	5	0.508	0.182	0.074
14	3190	1	0	vicariance	5	0.186	5	0.978	0.212	0.116
14	3190	1	0	dispersal	5	0.214	7	0.520	0.273	0.430
14	3190	1	0.1	dispersal	6	0.330	5	0.516	0.182	0.080
15	654	0.25	0	parapatric	8	1.000	4	0.300	0.242	0.236
15	654	0.25	0	vicariance	8	1.000	7	0.988	0.379	0.874
15	654	0.25	0.1	dispersal	8	1.000	6	1.000	0.364	0.954
15	654	0.5	0	parapatric	5	0.212	4	0.406	0.152	0.052
15	654	0.5	0	vicariance	5	0.206	6	1.000	0.212	0.128
15	654	0.5	0	dispersal	5	0.152	8	0.074	0.303	0.464
15	654	0.5	0.1	dispersal	6	0.330	6	1.000	0.265	0.268
15	654	0.75	0.1	dispersal	4	0.066	6	1.000	0.182	0.072
15	1309	0.25	0	parapatric	9	0.634	4	0.294	0.273	0.444
15	1309	0.25	0	vicariance	9	0.628	6	1.000	0.455	0.502
15	1309	0.25	0.1	dispersal	9	0.688	6	1.000	0.379	0.954
15	1309	0.5	0	parapatric	7	0.672	4	0.326	0.182	0.100
15	1309	0.5	0	vicariance	7	0.632	5	0.966	0.265	0.326
15	1309	0.5	0	dispersal	7	0.688	8	0.096	0.371	0.938
15	1309	0.5	0.1	dispersal	7	0.752	6	1.000	0.273	0.420
15	1309	0.75	0	vicariance	5	0.184	5	0.850	0.182	0.070
15	1309	0.75	0	dispersal	5	0.148	8	0.192	0.273	0.390
15	1309	0.75	0.1	dispersal	5	0.296	6	1.000	0.227	0.152
15	1309	1	0.1	dispersal	4	0.086	6	1.000	0.182	0.052
15	1595	0.25	0	parapatric	10	0.460	4	0.258	0.273	0.466
15	1595	0.25	0	vicariance	10	0.512	7	0.988	0.477	0.438
15	1595	0.25	0.1	dispersal	10	0.538	5	0.838	0.375	0.980
15	1595	0.5	0	parapatric	7	0.882	4	0.302	0.205	0.150
15	1595	0.5	0	vicariance	7	0.828	5	0.938	0.273	0.424
15	1595	0.5	0	dispersal	7	0.812	8	0.132	0.424	0.818
15	1595	0.5	0.1	dispersal	7	0.918	5	0.912	0.273	0.478
15	1595	0.75	0	vicariance	5	0.192	5	0.806	0.189	0.108
15	1595	0.75	0	dispersal	5	0.254	8	0.278	0.303	0.490
15	1595	0.75	0.1	dispersal	6	0.408	5	0.958	0.227	0.198
15	1595	1	0	vicariance	4	0.060	5	0.800	0.152	0.052
15	1595	1	0	dispersal	4	0.064	8	0.396	0.227	0.182
15	1595	1	0.1	dispersal	5	0.124	5	0.912	0.182	0.056
15	3190	0.25	0	parapatric	11	0.202	4	0.286	0.303	0.656
15	3190	0.25	0	vicariance	11	0.192	6	1.000	0.500	0.240
15	3190	0.25	0.1	dispersal	11	0.260	4	0.196	0.295	0.462
15	3190	0.5	0	parapatric	8	1.000	4	0.272	0.242	0.270
15	3190	0.5	0	vicariance	8	1.000	6	1.000	0.341	0.774
15	3190	0.5	0	dispersal	8.5	1.000	7	0.360	0.477	0.480
15	3190	0.5	0.1	dispersal	9	0.990	4	0.202	0.227	0.246
15	3190	0.75	0	parapatric	7	0.710	4	0.322	0.205	0.128
15	3190	0.75	0	vicariance	7	0.740	5	0.680	0.242	0.222
15	3190	0.75	0	dispersal	7	0.748	7	0.620	0.364	0.864
15	3190	0.75	0.1	dispersal	7	0.816	4	0.266	0.205	0.126
15	3190	1	0	vicariance	5	0.296	5	0.672	0.189	0.106
15	3190	1	0	dispersal	6	0.296	7	0.900	0.273	0.406
16	654	0.25	0	parapatric	9	0.950	3	0.126	0.212	0.194
16	654	0.25	0	vicariance	9	0.940	6	1.000	0.364	0.936
16	654	0.25	0	dispersal	9	0.966	7	0.190	0.424	0.338
16	654	0.25	0.1	dispersal	9	0.958	5	0.928	0.341	0.802

16	654	0.5	0	vicariance	6	0.322	5	0.680	0.189	0.072
16	654	0.5	0	dispersal	5	0.286	7	0.366	0.273	0.488
16	654	0.5	0.1	dispersal	6	0.464	5	0.958	0.227	0.204
16	654	0.75	0.1	dispersal	5	0.130	5	0.910	0.182	0.056
16	1309	0.25	0	parapatric	10	0.418	4	0.122	0.273	0.374
16	1309	0.25	0	vicariance	10	0.474	6	1.000	0.417	0.676
16	1309	0.25	0	dispersal	10	0.438	7	0.300	0.530	0.126
16	1309	0.25	0.1	dispersal	10	0.470	5	0.566	0.364	0.888
16	1309	0.5	0	parapatric	7	0.882	4	0.144	0.182	0.066
16	1309	0.5	0	vicariance	7	0.774	5	0.542	0.242	0.230
16	1309	0.5	0	dispersal	7	0.826	7	0.542	0.371	0.988
16	1309	0.5	0.1	dispersal	7	0.992	5	0.606	0.265	0.296
16	1309	0.75	0	vicariance	5	0.260	5	0.522	0.182	0.090
16	1309	0.75	0	dispersal	5	0.234	7	0.652	0.265	0.362
16	1309	0.75	0.1	dispersal	6	0.382	5	0.626	0.212	0.092
16	1309	1	0	dispersal	4	0.050	7	0.668	0.212	0.114
16	1595	0.25	0	parapatric	10	0.328	3	0.126	0.273	0.404
16	1595	0.25	0	vicariance	10	0.344	6	1.000	0.417	0.614
16	1595	0.25	0	dispersal	10	0.340	7	0.364	0.530	0.114
16	1595	0.25	0.1	dispersal	10	0.382	5	0.478	0.341	0.844
16	1595	0.5	0	parapatric	8	1.000	3	0.148	0.182	0.114
16	1595	0.5	0	vicariance	7	0.976	5	0.562	0.265	0.300
16	1595	0.5	0	dispersal	8	1.000	7	0.612	0.371	0.908
16	1595	0.5	0.1	dispersal	8	1.000	5	0.486	0.265	0.290
16	1595	0.75	0	vicariance	6	0.350	4	0.508	0.182	0.074
16	1595	0.75	0	dispersal	6	0.338	7	0.768	0.273	0.428
16	1595	0.75	0.1	dispersal	6	0.434	5	0.436	0.208	0.094
16	1595	1	0	dispersal	4	0.090	7	0.804	0.212	0.138
16	3190	0.25	0	parapatric	12	0.098	3	0.138	0.295	0.530
16	3190	0.25	0	vicariance	11	0.128	6	1.000	0.477	0.398
16	3190	0.25	0	dispersal	12	0.096	7	0.608	0.583	0.056
16	3190	0.25	0.1	dispersal	11	0.120	3	0.072	0.273	0.380
16	3190	0.5	0	parapatric	9	0.816	4	0.112	0.227	0.226
16	3190	0.5	0	vicariance	9	0.836	5	0.576	0.318	0.626
16	3190	0.5	0	dispersal	9	0.798	6	1.000	0.424	0.580
16	3190	0.5	0.1	dispersal	9	0.764	3	0.064	0.212	0.128
16	3190	0.75	0	parapatric	7	0.892	3	0.146	0.182	0.080
16	3190	0.75	0	vicariance	7	0.916	4	0.404	0.227	0.208
16	3190	0.75	0	dispersal	7	0.912	6	1.000	0.318	0.654
16	3190	0.75	0.1	dispersal	7	0.968	4	0.090	0.186	0.084
16	3190	1	0	vicariance	6	0.378	4	0.370	0.182	0.074
16	3190	1	0	dispersal	6	0.414	6	1.000	0.265	0.230
16	3190	1.5	0	dispersal	4	0.076	6	1.000	0.182	0.062
17	654	0.25	0	vicariance	9	0.806	5	0.492	0.318	0.636
17	654	0.25	0	dispersal	9	0.758	6	1.000	0.409	0.784
17	654	0.25	0.1	dispersal	9	0.770	5	0.384	0.303	0.578
17	654	0.5	0	vicariance	6	0.432	4	0.292	0.182	0.062
17	654	0.5	0	dispersal	6	0.378	6	1.000	0.265	0.294
17	654	0.5	0.1	dispersal	6	0.598	5	0.384	0.227	0.102
17	654	0.75	0	dispersal	4	0.050	7	0.984	0.182	0.084
17	1309	0.25	0	vicariance	11	0.292	5	0.522	0.379	0.914
17	1309	0.25	0	dispersal	10	0.278	6	1.000	0.455	0.322
17	1309	0.25	0.1	dispersal	10.5	0.306	4	0.208	0.326	0.682
17	1309	0.5	0	parapatric	8	1.000	3	0.054	0.159	0.064
17	1309	0.5	0	vicariance	8	1.000	4	0.224	0.227	0.164
17	1309	0.5	0	dispersal	7	0.996	6	1.000	0.318	0.604

17	1309	0.5	0.1	dispersal	8	1.000	4	0.202	0.242	0.156
17	1309	0.75	0	dispersal	6	0.350	6	1.000	0.242	0.184
17	1309	1	0	dispersal	4	0.102	6	1.000	0.189	0.102
17	1595	0.25	0	vicariance	11	0.220	5	0.570	0.394	0.830
17	1595	0.25	0	dispersal	11	0.212	6	1.000	0.500	0.248
17	1595	0.25	0.1	dispersal	11	0.228	4	0.136	0.303	0.642
17	1595	0.5	0	vicariance	8	1.000	4	0.202	0.242	0.204
17	1595	0.5	0	dispersal	8	1.000	6	1.000	0.364	0.726
17	1595	0.5	0.1	dispersal	8	1.000	4	0.128	0.242	0.202
17	1595	0.75	0	dispersal	6	0.432	6	1.000	0.265	0.188
17	1595	0.75	0.1	dispersal	7	0.658	4	0.158	0.189	0.056
17	1595	1	0	dispersal	5	0.116	6	1.000	0.189	0.086
17	3190	0.25	0	vicariance	12	0.070	5	0.496	0.455	0.508
17	3190	0.25	0	dispersal	12	0.052	6	1.000	0.530	0.122
17	3190	0.5	0	vicariance	9	0.636	4	0.190	0.273	0.424
17	3190	0.5	0	dispersal	10	0.608	6	1.000	0.409	0.860
17	3190	0.75	0	vicariance	8	1.000	4	0.138	0.212	0.118
17	3190	0.75	0	dispersal	8	1.000	5	0.772	0.303	0.460
17	3190	1	0	dispersal	6	0.570	5	0.658	0.227	0.122
18	654	0.5	0	dispersal	6	0.514	5	0.526	0.227	0.144
18	1309	0.5	0	dispersal	8	1.000	5	0.158	0.303	0.404
18	1309	0.75	0	dispersal	6	0.438	5	0.496	0.227	0.090
18	1595	0.5	0	dispersal	9	0.998	5	0.124	0.303	0.516
18	1595	0.75	0	dispersal	6	0.564	5	0.408	0.227	0.122
18	1595	1	0	dispersal	5	0.146	5	0.640	0.182	0.052
18	3190	0.75	0	dispersal	8	1.000	4	0.086	0.265	0.260
18	3190	1	0	dispersal	7	0.668	4	0.160	0.212	0.068

Table A.2.7. Parameter values for simulations of strict biological species where the observed data frequently arose ($p \geq 0.05$ for all three indices). CO = complete overlap, ZO = zero overlap.

Sympatric speciation events	Starting range size	Range movements	Range growth	Allopatric mode of speciation	Median cases of CO	Probability of observed CO	Median cases of ZO	Probability of observed ZO	Median bimodality	Probability of observed bimodality
9	2680	0.25	0	parapatric	6	0.060	6	0.174	0.318	0.518
9	2680	0.25	0.1	dispersal	6	0.054	6	0.164	0.318	0.556
9	3515	0.25	0	parapatric	7	0.094	6	0.122	0.327	0.376
9	3515	0.25	0.1	dispersal	6	0.092	4	0.556	0.250	0.980
10	1340	0.25	0	parapatric	6	0.098	6	0.206	0.291	0.598
10	1757	0.25	0	parapatric	6	0.160	5	0.248	0.318	0.568
10	2680	0.25	0	parapatric	7	0.260	6	0.212	0.327	0.428
10	2680	0.25	0.1	dispersal	7	0.212	5	0.248	0.318	0.544
10	3515	0.25	0	parapatric	7	0.354	6	0.228	0.364	0.362
10	3515	0.25	0.1	dispersal	7	0.306	4	0.750	0.245	0.938
10	3515	0.5	0	parapatric	6	0.052	5	0.232	0.273	0.812
10	3515	0.5	0.1	dispersal	6	0.056	4	0.574	0.218	0.796
11	1340	0.25	0	parapatric	7	0.266	5	0.336	0.318	0.622
11	1757	0.25	0	parapatric	7	0.334	5	0.364	0.318	0.560
11	1757	0.25	0.1	dispersal	7	0.338	6	0.090	0.364	0.274
11	2680	0.25	0	parapatric	8	0.564	5	0.396	0.327	0.472
11	2680	0.25	0.1	dispersal	8	0.504	5	0.354	0.318	0.472
11	2680	0.5	0	parapatric	6	0.088	5	0.336	0.255	0.968
11	2680	0.5	0	vicariance	6	0.090	7	0.066	0.327	0.488
11	2680	0.5	0.1	dispersal	6	0.108	5	0.314	0.255	0.938
11	3515	0.25	0	parapatric	8	0.674	5	0.324	0.364	0.376
11	3515	0.25	0.1	dispersal	8	0.618	4	0.836	0.255	0.844
11	3515	0.5	0	parapatric	6	0.152	5	0.338	0.273	0.814

11	3515	0.5	0.1	dispersal	6	0.150	4	0.738	0.218	0.776
12	1340	0.25	0	parapatric	7	0.482	4	0.516	0.273	0.690
12	1340	0.25	0.1	dispersal	7	0.470	6	0.100	0.382	0.238
12	1340	0.5	0	parapatric	5	0.052	5	0.484	0.191	0.710
12	1340	0.5	0	vicariance	5	0.060	6	0.114	0.273	0.872
12	1340	0.5	0.1	dispersal	5	0.062	6	0.088	0.273	0.736
12	1757	0.25	0	parapatric	8	0.640	4	0.582	0.291	0.602
12	1757	0.25	0.1	dispersal	8	0.568	5	0.184	0.364	0.290
12	1757	0.5	0	parapatric	5	0.100	5	0.490	0.218	0.774
12	1757	0.5	0	vicariance	5	0.076	6	0.088	0.282	0.684
12	1757	0.5	0.1	dispersal	6	0.096	6	0.120	0.273	0.792
12	2680	0.25	0	parapatric	8	0.916	4	0.498	0.327	0.446
12	2680	0.25	0.1	dispersal	8	0.814	4	0.556	0.318	0.520
12	2680	0.5	0	parapatric	6	0.146	4.5	0.488	0.227	0.998
12	2680	0.5	0	vicariance	6	0.220	6	0.126	0.327	0.468
12	2680	0.5	0.1	dispersal	6	0.196	5	0.480	0.255	0.954
12	2680	0.75	0.1	dispersal	5	0.050	5	0.406	0.218	0.726
12	3515	0.25	0	parapatric	9	1.000	4	0.518	0.327	0.384
12	3515	0.25	0.1	dispersal	9	1.000	3	1.000	0.245	0.908
12	3515	0.5	0	parapatric	7	0.280	4	0.510	0.273	0.826
12	3515	0.5	0	vicariance	7	0.264	6	0.120	0.364	0.424
12	3515	0.5	0.1	dispersal	7	0.304	4	0.992	0.218	0.780
12	3515	0.75	0	parapatric	5	0.080	4	0.520	0.218	0.726
12	3515	0.75	0	vicariance	5	0.078	6	0.170	0.255	0.936
12	3515	0.75	0.1	dispersal	6	0.100	4	0.850	0.182	0.554
13	1340	0.25	0	parapatric	8	0.696	4	0.712	0.273	0.748
13	1340	0.25	0.1	dispersal	8	0.736	5	0.200	0.364	0.308
13	1340	0.5	0	parapatric	6	0.106	4	0.740	0.182	0.638
13	1340	0.5	0	vicariance	5	0.094	5	0.190	0.255	0.916
13	1340	0.5	0.1	dispersal	6	0.098	5	0.176	0.273	0.860
13	1757	0.25	0	parapatric	8	0.954	4	0.742	0.291	0.640
13	1757	0.25	0.1	dispersal	8	0.860	5	0.370	0.364	0.366
13	1757	0.5	0	parapatric	6	0.148	4	0.770	0.218	0.748
13	1757	0.5	0	vicariance	6	0.164	5	0.204	0.273	0.794
13	1757	0.5	0.1	dispersal	6	0.182	5	0.362	0.273	0.858
13	2680	0.25	0	parapatric	9	1.000	4	0.730	0.318	0.514
13	2680	0.25	0.1	dispersal	9	1.000	4	0.764	0.300	0.528
13	2680	0.5	0	parapatric	7	0.364	4	0.694	0.227	0.988
13	2680	0.5	0	vicariance	7	0.316	5	0.212	0.318	0.574
13	2680	0.5	0.1	dispersal	7	0.378	4	0.736	0.236	1.000
13	2680	0.75	0	parapatric	5	0.074	4	0.752	0.182	0.522
13	2680	0.75	0	vicariance	5	0.072	5	0.328	0.227	0.868
13	2680	0.75	0.1	dispersal	5	0.130	4	0.632	0.191	0.662
13	2680	1	0.1	dispersal	5	0.056	4	0.674	0.164	0.418
13	3515	0.25	0	parapatric	9	1.000	4	0.768	0.327	0.482
13	3515	0.25	0.1	dispersal	9	1.000	3	1.000	0.245	0.994
13	3515	0.5	0	parapatric	7	0.488	4	0.682	0.255	0.846
13	3515	0.5	0	vicariance	7	0.446	5	0.194	0.327	0.474
13	3515	0.5	0.1	dispersal	7	0.486	3	1.000	0.191	0.756
13	3515	0.75	0	parapatric	6	0.112	4	0.750	0.191	0.690
13	3515	0.75	0	vicariance	6	0.120	5	0.362	0.255	0.974
13	3515	0.75	0.1	dispersal	6	0.190	3	1.000	0.182	0.486
13	3515	1	0.1	dispersal	5	0.052	3	1.000	0.145	0.346
14	1340	0.25	0	parapatric	9	1.000	3	1.000	0.255	0.870
14	1340	0.25	0	vicariance	9	1.000	6	0.096	0.436	0.146
14	1340	0.25	0.1	dispersal	8.5	1.000	5	0.392	0.327	0.346

14	1340	0.5	0	parapatric	6	0.156	3	1.000	0.182	0.564
14	1340	0.5	0	vicariance	6	0.138	5	0.380	0.227	0.964
14	1340	0.5	0.1	dispersal	6	0.222	5	0.360	0.255	0.930
14	1757	0.25	0	parapatric	9	1.000	4	0.936	0.291	0.724
14	1757	0.25	0	vicariance	9	1.000	6	0.092	0.436	0.100
14	1757	0.25	0.1	dispersal	9	1.000	4	0.562	0.327	0.412
14	1757	0.5	0	parapatric	6	0.264	4	0.974	0.191	0.698
14	1757	0.5	0	vicariance	6	0.232	5	0.384	0.255	0.856
14	1757	0.5	0.1	dispersal	7	0.326	4	0.534	0.255	0.914
14	1757	0.75	0	parapatric	5	0.052	4	0.930	0.145	0.332
14	1757	0.75	0.1	dispersal	5	0.080	4	0.510	0.182	0.586
14	2680	0.25	0	parapatric	10	0.980	3	1.000	0.291	0.640
14	2680	0.25	0	vicariance	10	0.942	6	0.108	0.455	0.056
14	2680	0.25	0.1	dispersal	9	1.000	3	1.000	0.291	0.656
14	2680	0.5	0	parapatric	7	0.536	3	1.000	0.218	0.880
14	2680	0.5	0	vicariance	7	0.528	5	0.408	0.295	0.588
14	2680	0.5	0.1	dispersal	7	0.502	3	1.000	0.218	0.894
14	2680	0.75	0	parapatric	6	0.114	4	0.996	0.164	0.538
14	2680	0.75	0	vicariance	5	0.096	4	0.532	0.218	0.726
14	2680	0.75	0.1	dispersal	6	0.208	4	0.966	0.182	0.580
14	2680	1	0.1	dispersal	5	0.056	4	0.914	0.145	0.362
14	3515	0.25	0	parapatric	10	0.802	3	1.000	0.300	0.596
14	3515	0.25	0	vicariance	10	0.754	6	0.102	0.491	0.052
14	3515	0.25	0.1	dispersal	10	0.874	3	1.000	0.218	0.914
14	3515	0.5	0	parapatric	8	0.740	4	0.984	0.245	0.946
14	3515	0.5	0	vicariance	8	0.698	5	0.382	0.318	0.500
14	3515	0.5	0.1	dispersal	8	0.642	3	1.000	0.191	0.594
14	3515	0.75	0	parapatric	6	0.216	4	0.988	0.191	0.640
14	3515	0.75	0	vicariance	6	0.206	4	0.558	0.227	0.904
14	3515	0.75	0.1	dispersal	6	0.292	3	1.000	0.164	0.432
14	3515	1	0	parapatric	5	0.054	4	0.958	0.164	0.362
14	3515	1	0	dispersal	5	0.074	6	0.114	0.236	1.000
14	3515	1	0.1	dispersal	5	0.100	3	1.000	0.136	0.286
15	1340	0.25	0	parapatric	9	1.000	3	1.000	0.245	0.922
15	1340	0.25	0	vicariance	9	1.000	5	0.244	0.400	0.198
15	1340	0.25	0.1	dispersal	9	1.000	4	0.698	0.309	0.472
15	1340	0.5	0	parapatric	6	0.262	3	1.000	0.164	0.492
15	1340	0.5	0	vicariance	6	0.240	4	0.678	0.218	0.810
15	1340	0.5	0.1	dispersal	6	0.292	4	0.632	0.218	0.918
15	1340	0.75	0.1	dispersal	5	0.066	4	0.666	0.182	0.454
15	1757	0.25	0	parapatric	9.5	1.000	3	1.000	0.245	0.870
15	1757	0.25	0	vicariance	10	0.978	5	0.244	0.409	0.138
15	1757	0.25	0.1	dispersal	10	0.964	4	0.924	0.300	0.582
15	1757	0.5	0	parapatric	7	0.420	3	1.000	0.182	0.584
15	1757	0.5	0	vicariance	7	0.412	4	0.694	0.245	0.976
15	1757	0.5	0.1	dispersal	7	0.476	4	0.874	0.218	0.906
15	1757	0.75	0	parapatric	5	0.090	3	1.000	0.136	0.242
15	1757	0.75	0	vicariance	5	0.054	4	0.850	0.164	0.362
15	1757	0.75	0.1	dispersal	6	0.124	4	0.848	0.182	0.480
15	2680	0.25	0	parapatric	10	0.694	3	1.000	0.264	0.842
15	2680	0.25	0	vicariance	10	0.630	5	0.268	0.436	0.090
15	2680	0.25	0.1	dispersal	10	0.734	3	1.000	0.255	0.862
15	2680	0.5	0	parapatric	8	0.790	3	1.000	0.218	0.804
15	2680	0.5	0	vicariance	8	0.696	4	0.696	0.273	0.768
15	2680	0.5	0.1	dispersal	8	0.800	3	1.000	0.218	0.868
15	2680	0.75	0	parapatric	6	0.210	3	1.000	0.145	0.412

15	2680	0.75	0	vicariance	6	0.222	4	0.880	0.182	0.642
15	2680	0.75	0	dispersal	6	0.184	5	0.078	0.273	0.714
15	2680	0.75	0.1	dispersal	6	0.216	3	1.000	0.164	0.450
15	2680	1	0	parapatric	5	0.076	3	1.000	0.127	0.208
15	2680	1	0	dispersal	5	0.054	5	0.176	0.218	0.726
15	2680	1	0.1	dispersal	5	0.106	3	1.000	0.145	0.308
15	3515	0.25	0	parapatric	11	0.516	3	1.000	0.273	0.784
15	3515	0.25	0	vicariance	11	0.514	5	0.276	0.436	0.088
15	3515	0.25	0.1	dispersal	11	0.558	2	0.832	0.218	0.758
15	3515	0.5	0	parapatric	8	0.954	3	1.000	0.218	0.868
15	3515	0.5	0	vicariance	8	0.876	4	0.702	0.291	0.658
15	3515	0.5	0	dispersal	8	0.966	5	0.064	0.409	0.148
15	3515	0.5	0.1	dispersal	8	0.904	2	0.918	0.182	0.546
15	3515	0.75	0	parapatric	7	0.346	3	1.000	0.182	0.536
15	3515	0.75	0	vicariance	7	0.344	4	0.896	0.218	0.788
15	3515	0.75	0	dispersal	7	0.318	5	0.178	0.291	0.618
15	3515	0.75	0.1	dispersal	7	0.392	2	0.998	0.145	0.338
15	3515	1	0	parapatric	5	0.082	3	1.000	0.136	0.292
15	3515	1	0	vicariance	5	0.090	4	0.906	0.164	0.456
15	3515	1	0	dispersal	5	0.098	5	0.316	0.218	0.820
15	3515	1	0.1	dispersal	6	0.178	3	1.000	0.136	0.240
16	1340	0.25	0	parapatric	10	0.888	3	1.000	0.218	0.836
16	1340	0.25	0	vicariance	10	0.902	4	0.594	0.341	0.318
16	1340	0.25	0.1	dispersal	10	0.864	3	1.000	0.291	0.654
16	1340	0.5	0	parapatric	7	0.394	3	1.000	0.145	0.372
16	1340	0.5	0	vicariance	7	0.400	3	1.000	0.191	0.680
16	1340	0.5	0	dispersal	7	0.386	5	0.054	0.291	0.572
16	1340	0.5	0.1	dispersal	7	0.470	3	1.000	0.191	0.724
16	1340	0.75	0	vicariance	5	0.056	3	1.000	0.136	0.228
16	1340	0.75	0.1	dispersal	5	0.106	3	1.000	0.164	0.328
16	1757	0.25	0	parapatric	10	0.662	3	1.000	0.218	0.902
16	1757	0.25	0	vicariance	10	0.694	4	0.548	0.364	0.262
16	1757	0.25	0.1	dispersal	10	0.740	3	1.000	0.273	0.774
16	1757	0.5	0	parapatric	7	0.594	3	1.000	0.164	0.450
16	1757	0.5	0	vicariance	7	0.576	3	1.000	0.218	0.822
16	1757	0.5	0	dispersal	7	0.584	5	0.060	0.318	0.448
16	1757	0.5	0.1	dispersal	8	0.692	3	1.000	0.191	0.732
16	1757	0.75	0	parapatric	5	0.122	3	1.000	0.109	0.208
16	1757	0.75	0	vicariance	5	0.110	3	1.000	0.145	0.314
16	1757	0.75	0	dispersal	5	0.126	5	0.124	0.227	0.856
16	1757	0.75	0.1	dispersal	6	0.202	3	1.000	0.164	0.368
16	2680	0.25	0	parapatric	11	0.458	3	1.000	0.236	0.992
16	2680	0.25	0	vicariance	11	0.438	4	0.668	0.364	0.214
16	2680	0.25	0.1	dispersal	11	0.484	2	0.932	0.236	0.894
16	2680	0.5	0	parapatric	8	0.896	3	1.000	0.182	0.612
16	2680	0.5	0	vicariance	8	0.926	3	1.000	0.245	0.962
16	2680	0.5	0	dispersal	8	0.930	5	0.134	0.364	0.252
16	2680	0.5	0.1	dispersal	8	0.914	3	1.000	0.191	0.648
16	2680	0.75	0	parapatric	6	0.350	3	1.000	0.145	0.314
16	2680	0.75	0	vicariance	6	0.290	3	1.000	0.182	0.528
16	2680	0.75	0	dispersal	6	0.306	4	0.284	0.255	0.944
16	2680	0.75	0.1	dispersal	7	0.368	3	1.000	0.145	0.348
16	2680	1	0	parapatric	5	0.084	3	1.000	0.109	0.188
16	2680	1	0	vicariance	5	0.070	3	1.000	0.136	0.230
16	2680	1	0	dispersal	5	0.076	4	0.368	0.182	0.580
16	2680	1	0.1	dispersal	6	0.140	3	1.000	0.109	0.188

16	3515	0.25	0	parapatric	11	0.278	2	0.992	0.245	0.978
16	3515	0.25	0	vicariance	11	0.310	4	0.626	0.400	0.158
16	3515	0.25	0.1	dispersal	11	0.372	2	0.498	0.191	0.524
16	3515	0.5	0	parapatric	9	1.000	2	0.946	0.182	0.664
16	3515	0.5	0	vicariance	9	1.000	3	1.000	0.255	0.814
16	3515	0.5	0	dispersal	9	1.000	5	0.230	0.364	0.212
16	3515	0.5	0.1	dispersal	9	1.000	2	0.610	0.145	0.394
16	3515	0.75	0	parapatric	7	0.496	3	1.000	0.164	0.426
16	3515	0.75	0	vicariance	7	0.468	3	1.000	0.182	0.624
16	3515	0.75	0	dispersal	7	0.470	4	0.452	0.273	0.780
16	3515	0.75	0.1	dispersal	7	0.550	2	0.724	0.136	0.328
16	3515	1	0	parapatric	6	0.188	3	1.000	0.136	0.226
16	3515	1	0	vicariance	6	0.164	3	1.000	0.145	0.370
16	3515	1	0	dispersal	6	0.172	4	0.640	0.191	0.668
16	3515	1	0.1	dispersal	6	0.270	2	0.692	0.109	0.142
17	1340	0.25	0	parapatric	10	0.676	2	0.646	0.191	0.604
17	1340	0.25	0	vicariance	10	0.638	3	1.000	0.291	0.626
17	1340	0.25	0	dispersal	10	0.716	4	0.178	0.364	0.094
17	1340	0.25	0.1	dispersal	10	0.736	3	1.000	0.236	0.966
17	1340	0.5	0	parapatric	7	0.514	2	0.678	0.127	0.232
17	1340	0.5	0	vicariance	7	0.530	3	1.000	0.164	0.450
17	1340	0.5	0	dispersal	7	0.532	4	0.402	0.255	0.898
17	1340	0.5	0.1	dispersal	8	0.680	3	1.000	0.164	0.506
17	1340	0.75	0	parapatric	5	0.088	2	0.790	0.091	0.098
17	1340	0.75	0	vicariance	5	0.082	3	1.000	0.127	0.186
17	1340	0.75	0	dispersal	5	0.090	4	0.418	0.182	0.456
17	1340	0.75	0.1	dispersal	6	0.146	3	1.000	0.127	0.190
17	1757	0.25	0	parapatric	11	0.476	2	0.638	0.200	0.646
17	1757	0.25	0	vicariance	11	0.512	3	1.000	0.327	0.494
17	1757	0.25	0	dispersal	11	0.518	4	0.260	0.382	0.060
17	1757	0.25	0.1	dispersal	11	0.536	2	0.944	0.218	0.882
17	1757	0.5	0	parapatric	8	0.738	2	0.622	0.127	0.280
17	1757	0.5	0	vicariance	8	0.720	3	1.000	0.182	0.580
17	1757	0.5	0	dispersal	8	0.754	4	0.472	0.255	0.674
17	1757	0.5	0.1	dispersal	8	0.804	3	1.000	0.164	0.534
17	1757	0.75	0	parapatric	6	0.168	2	0.756	0.109	0.144
17	1757	0.75	0	vicariance	6	0.160	3	1.000	0.127	0.202
17	1757	0.75	0	dispersal	6	0.148	4	0.550	0.191	0.612
17	1757	0.75	0.1	dispersal	6	0.290	2	0.978	0.136	0.186
17	1757	1	0.1	dispersal	5	0.084	3	1.000	0.109	0.088
17	2680	0.25	0	parapatric	12	0.270	2	0.652	0.218	0.732
17	2680	0.25	0	vicariance	12	0.272	3	1.000	0.327	0.482
17	2680	0.25	0.1	dispersal	11	0.330	2	0.564	0.200	0.596
17	2680	0.5	0	parapatric	9	1.000	2	0.666	0.164	0.448
17	2680	0.5	0	vicariance	9	1.000	3	1.000	0.191	0.716
17	2680	0.5	0	dispersal	9	1.000	4	0.608	0.291	0.506
17	2680	0.5	0.1	dispersal	9	1.000	2	0.564	0.164	0.352
17	2680	0.75	0	parapatric	7	0.452	2	0.652	0.109	0.188
17	2680	0.75	0	vicariance	7	0.374	3	1.000	0.145	0.316
17	2680	0.75	0	dispersal	7	0.414	4	0.818	0.218	0.818
17	2680	0.75	0.1	dispersal	7	0.514	2	0.634	0.127	0.192
17	2680	1	0	parapatric	5	0.090	2	0.840	0.109	0.108
17	2680	1	0	vicariance	5	0.106	3	1.000	0.109	0.134
17	2680	1	0	dispersal	5	0.106	4	0.922	0.164	0.396
17	2680	1	0.1	dispersal	6	0.214	2	0.704	0.109	0.106
17	3515	0.25	0	parapatric	12	0.180	2	0.624	0.218	0.790

17	3515	0.25	0	vicariance	12	0.164	3	1.000	0.327	0.388
17	3515	0.25	0	dispersal	12	0.180	4	0.460	0.400	0.052
17	3515	0.25	0.1	dispersal	12	0.254	1	0.262	0.127	0.340
17	3515	0.5	0	parapatric	10	0.970	2	0.608	0.164	0.478
17	3515	0.5	0	vicariance	9	1.000	3	1.000	0.218	0.926
17	3515	0.5	0	dispersal	10	0.992	4	0.800	0.295	0.470
17	3515	0.5	0.1	dispersal	9	1.000	2	0.326	0.109	0.228
17	3515	0.75	0	parapatric	8	0.686	2	0.636	0.136	0.236
17	3515	0.75	0	vicariance	8	0.650	3	1.000	0.164	0.422
17	3515	0.75	0	dispersal	7	0.620	3	1.000	0.218	0.876
17	3515	0.75	0.1	dispersal	8	0.690	2	0.378	0.105	0.120
17	3515	1	0	parapatric	6	0.198	2	0.772	0.109	0.166
17	3515	1	0	vicariance	6	0.222	2	0.982	0.127	0.210
17	3515	1	0	dispersal	6	0.208	3	1.000	0.164	0.410
17	3515	1	0.1	dispersal	7	0.300	2	0.422	0.091	0.084
18	1340	0.25	0	parapatric	11	0.520	1	0.226	0.127	0.266
18	1340	0.25	0	vicariance	11	0.454	2	0.926	0.236	0.932
18	1340	0.25	0	dispersal	11	0.466	3	1.000	0.300	0.342
18	1340	0.25	0.1	dispersal	11	0.482	2	0.532	0.200	0.526
18	1340	0.5	0	parapatric	8	0.658	2	0.316	0.091	0.128
18	1340	0.5	0	vicariance	8	0.656	2	0.580	0.136	0.192
18	1340	0.5	0	dispersal	8	0.698	3	1.000	0.195	0.664
18	1340	0.5	0.1	dispersal	8	0.772	2	0.598	0.145	0.216
18	1340	0.75	0	parapatric	5	0.124	2	0.544	0.082	0.052
18	1340	0.75	0	vicariance	5	0.138	2	0.736	0.109	0.082
18	1340	0.75	0	dispersal	5	0.116	3	1.000	0.145	0.214
18	1340	0.75	0.1	dispersal	6	0.236	2	0.588	0.109	0.074
18	1757	0.25	0	parapatric	12	0.340	2	0.294	0.145	0.380
18	1757	0.25	0	vicariance	12	0.294	2	0.930	0.245	0.992
18	1757	0.25	0	dispersal	12	0.346	3	1.000	0.300	0.288
18	1757	0.25	0.1	dispersal	11	0.350	2	0.426	0.191	0.520
18	1757	0.5	0	parapatric	8	0.928	2	0.292	0.109	0.138
18	1757	0.5	0	vicariance	8	0.884	2	0.572	0.145	0.268
18	1757	0.5	0	dispersal	8	0.880	3	1.000	0.218	0.766
18	1757	0.5	0.1	dispersal	8	0.988	2	0.458	0.136	0.204
18	1757	0.75	0	parapatric	6	0.246	2	0.408	0.091	0.086
18	1757	0.75	0	vicariance	6	0.242	2	0.676	0.109	0.118
18	1757	0.75	0	dispersal	6	0.234	3	1.000	0.164	0.294
18	1757	0.75	0.1	dispersal	7	0.352	2	0.502	0.109	0.080
18	1757	1	0	vicariance	5	0.076	2	0.848	0.091	0.074
18	2680	0.25	0	parapatric	12	0.172	1	0.258	0.145	0.430
18	2680	0.25	0	vicariance	12	0.152	2	0.862	0.255	0.906
18	2680	0.25	0	dispersal	13	0.144	3	1.000	0.327	0.230
18	2680	0.25	0.1	dispersal	12	0.210	1	0.206	0.136	0.364
18	2680	0.5	0	parapatric	9	1.000	1	0.306	0.109	0.184
18	2680	0.5	0	vicariance	9	1.000	2	0.548	0.164	0.388
18	2680	0.5	0	dispersal	9	1.000	3	1.000	0.245	0.982
18	2680	0.5	0.1	dispersal	9	1.000	1	0.240	0.109	0.164
18	2680	0.75	0	parapatric	7	0.574	2	0.332	0.091	0.096
18	2680	0.75	0	vicariance	7	0.556	2	0.526	0.127	0.152
18	2680	0.75	0	dispersal	7	0.500	3	1.000	0.164	0.358
18	2680	0.75	0.1	dispersal	8	0.632	2	0.280	0.100	0.076
18	2680	1	0	parapatric	6	0.142	2	0.496	0.082	0.068
18	2680	1	0	vicariance	6	0.150	2	0.638	0.091	0.076
18	2680	1	0	dispersal	6	0.154	3	1.000	0.136	0.184
18	2680	1	0.1	dispersal	6	0.276	2	0.314	0.082	0.056

18	3515	0.25	0	parapatric	13	0.072	1	0.226	0.145	0.500
18	3515	0.25	0	vicariance	13	0.132	2	0.908	0.255	0.812
18	3515	0.25	0	dispersal	13	0.090	3	1.000	0.327	0.272
18	3515	0.25	0.1	dispersal	12	0.126	1	0.070	0.118	0.258
18	3515	0.5	0	parapatric	10	0.820	1	0.252	0.118	0.248
18	3515	0.5	0	vicariance	10	0.800	2	0.596	0.182	0.456
18	3515	0.5	0	dispersal	10	0.814	3	1.000	0.245	0.936
18	3515	0.5	0.1	dispersal	10	0.860	1	0.096	0.100	0.080
18	3515	0.75	0	parapatric	8	0.796	2	0.334	0.109	0.122
18	3515	0.75	0	vicariance	8	0.758	2	0.474	0.127	0.170
18	3515	0.75	0	dispersal	8	0.756	3	1.000	0.164	0.466
18	3515	1	0	vicariance	6	0.324	2	0.548	0.109	0.112
18	3515	1	0	dispersal	6	0.292	3	1.000	0.136	0.190
19	1340	0.75	0	dispersal	6	0.176	2	0.662	0.109	0.104
19	1757	0.5	0	dispersal	9	1.000	2	0.124	0.145	0.102
19	1757	0.75	0	dispersal	6	0.340	2	0.394	0.109	0.066
19	1757	1	0	dispersal	5	0.062	2	0.880	0.109	0.072
19	2680	0.5	0	dispersal	10	0.820	2	0.060	0.164	0.120
19	2680	0.75	0	dispersal	8	0.680	2	0.200	0.127	0.052
19	2680	1	0	dispersal	6	0.198	2	0.494	0.109	0.070
19	3515	0.75	0	dispersal	8	0.940	2	0.108	0.127	0.050
19	3515	1	0	dispersal	7	0.402	2	0.304	0.109	0.064

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